Relationship of Glucose Metabolism to Adrenergic Transmission in Rat Mesenteric Arteries

EFFECTS OF GLUCOSE DEPRIVATION, GLUCOSE METABOLITES, AND CHANGES IN IONIC COMPOSITION ON ADRENERGIC MECHANISMS

By Kafait U. Malik and John C. McGiff

ABSTRACT
The vasoconstrictor response of perfused rat mesenteric arteries to stimulation of sympathetic nerve fibers is markedly potentiated by glucose deprivation; this potentiation is abolished or reduced when glucose or other sugars are added. The augmentation of the vasoconstrictor response to nerve stimulation produced by glucose deprivation presumably results from an increased release of the adrenergic transmitter, since (1) the response to injected norepinephrine is much less affected by glucose deprivation and (2) the increase in the vasoconstrictor response to either adrenergic stimulus produced by inhibition of neuronal reuptake by cocaine is unaltered by glucose deprivation. The inhibitory effect of glucose may involve its metabolite(s). Pyruvic and lactic acids inhibit the vasoconstrictor response to nerve stimulation previously augmented by glucose deprivation but do not affect adrenergic transmission in the presence of glucose. Also, the inhibitory effect of glucose on the potentiated response is abolished by the simultaneous infusion of 2-deoxy-D-glucose or iodoacetic acid, inhibitors of glucose metabolism. The inhibitory effect of glucose and its metabolite(s) on adrenergic transmission may also involve changes in the ionic permeability of the nerve terminal. In the absence of glucose, raising the Na⁺ and K⁺ concentrations affects the vasoconstrictor response differently, namely, Na⁺ potentiates and K⁺ attenuates the response. These effects are abolished by addition of glucose. In contrast, the effects of increased concentrations of either Ca²⁺ (facilitation) or Mg²⁺ (inhibition) on neurotransmission are unaffected by removal or restoration of glucose. We conclude that glucose deprivation does not affect adrenergic transmission by acting directly through Ca²⁺. Rather, glucose deprivation decreases pyruvate and possibly other products of glucose metabolism, and these decreases, in turn, alter the concentrations of Na⁺ and K⁺ within the neuron. These latter changes then enhance the availability of Ca²⁺ and, thereby, increase the release of the adrenergic transmitter.

KEY WORDS
mesenteric vasculature sympathetic transmission
potentiation of vasoconstriction adrenergic release mechanism
pyruvic acid vascular reactivity adenine nucleotides
2-deoxy-D-glucose iodoacetic acid prostaglandins electrolytes

Glucose deprivation causes early failure of transmission in the perfused cat superior cervical ganglion during prolonged stimulation; this transmission failure is prevented by addition of glucose to the perfusion medium (1). Larrabee and Bronk (2) have reported that glucose deprivation induces a more rapid decline in the action potentials of the postganglionic fibers than it does in those of the preganglionic fibers of the rat superior cervical ganglion, thereby suggesting that synaptic transmission is more vulnerable to lack of glucose than is axonal conduction. Nicolescu et al. (3) have shown that synaptic transmission is lost within 2.5 hours after glucose withdrawal whereas axonal conduction is still measurable in the postganglionic nerve for more than 24 hours after glucose deprivation. Moreover, the loss of synaptic transmission is accompanied by lesions of the presynaptic fibers, but postganglionic fibers show no damage. Although glucose deprivation causes failure of synaptic transmission in the ganglion and decline of action potentials in postganglionic sympathetic
fibers, it does not affect the release of catecholamines from the adrenal glands evoked by acetylcholine (4) or from the cat spleen produced by stimulation of postganglionic sympathetic fibers (5). However, exclusion of glucose from the fluid superfusing the isolated rat iris previously incubated with tritiated norepinephrine increases both spontaneous and field stimulation-induced overflow of tritiated norepinephrine (6).

In the present study, we found a marked glucose deprivation-induced potentiation of isolated perfused rat mesenteric arteries to postganglionic sympathetic nerve stimulation. To elucidate the mechanism of facilitation of adrenergic transmission produced by glucose deprivation, we examined (1) the norepinephrine content of mesenteric vessels during stimulation of their sympathetic nerve fibers in the presence and the absence of glucose, (2) the effects of several glucose metabolites and inhibitors of glucose metabolism on the potentiated vasoconstrictor response, (3) the possible participation of autonomic regulatory mechanisms and prostaglandins in the inhibitory action of glucose on adrenergic transmission in the mesenteric arteries, and (4) the effects of changes in the ionic composition of the perfusion fluid on the potentiated vasoconstrictor responses to nerve stimulation. The effects of other hexoses, fructose, mannose, and galactose, and a pentose, xylose, on the vasoconstrictor responses to adrenergic stimuli were also studied to see if, like glucose, these substances inhibit adrenergic transmission in rat mesenteric arteries previously facilitated by glucose deprivation. The work of McGregor (7) and our previous observations (8) indicate that periarterial nerves to the rat mesenteric arteries are postganglionic adrenergic fibers; therefore, the effects of sugars and other agents on the vasoconstrictor response of the mesenteric arteries to nerve stimulation reported in this study are dependent on the action of the test substances on the adrenergic neuroeffector unit.

**Methods**

Female albino rats (300-350 g) were anesthetized with ether, and their abdomens were opened by a midline incision. The superior mesenteric artery was cannulated at a constant flow of 20 ml/min using a Harvard model 1210 peristaltic pump. The details of the method have been described previously (9, 10). The millimolar composition of the Tyrode's solution was: NaCl 137, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.1, NaHCO₃ 12, NaH₂PO₄ 0.42, d-glucose 5.6. The temperature of the perfusion fluid in these experiments was maintained at 35°C or 22°C; the solution was aerated with a mixture of 95% O₂-5% CO₂. Changes in perfusion pressure were recorded manometrically by a frontal writing lever on a kymograph. Before cannulation of the superior mesenteric artery, the pressure in the cannula was 60 mm Hg at a flow rate of 20 ml/min. During perfusion of the artery, the pressure was 80 mm Hg.

The periarterial nerves were stimulated at 6 Hz for 22 seconds at 4-minute intervals with a Grass model S44 stimulator, using supramaximal biphasic rectangular pulses 1 msec in duration. Norepinephrine (0.5-3 μg) was injected directly into the arterial cannula. We compared the effects of any intervention on the mesenteric vasoconstrictor response to nerve stimulation with its effects on the response to a dose of injected norepinephrine that produced an equal degree of mesenteric vasoconstriction (equiconstrictor response). Therefore, we could distinguish between effects of an intervention on release of norepinephrine and effects on reuptake of norepinephrine or vascular reactivity. For example, if an intervention altered the mesenteric vasoconstrictor response to nerve stimulation but not the response to an equiconstrictor dose of norepinephrine, then it probably affected the release mechanism. If, on the other hand, a change in the response to injected norepinephrine occurred to a greater degree, then either reuptake of norepinephrine or vascular reactivity was primarily affected. All other drugs were added to Tyrode's solution with or without glucose as will be described later. The percent change in the vasoconstrictor response to nerve stimulation and to injected norepinephrine produced by various agents was calculated by comparing the mean height of vasoconstrictor responses during the infusion of an agent for 16-24 minutes with the mean height of control responses recorded in the absence of the drug.

The content of norepinephrine in the mesenteric arteries was determined fluorometrically using a modification of the semiautomated method of Merrills (11), which, in turn, is a modification of the original trihydroxyindole technique of Lund (12) used for whole blood samples. Mesenteric vessels were perfused with Tyrode's solution containing glucose (13 preparations) or with glucose-free Tyrode's solution (13 preparations), and their sympathetic fibers were stimulated at 6 Hz for 22 seconds at 4-minute intervals over a period of 90 minutes. The superior mesenteric artery, together with a part of its small resistance vessels, was then rapidly blotted and weighed; the average weight of the tissue samples was 500 mg. The tissue sample was transferred to a 12-ml glass Corex tube containing 2 ml of 0.4N HClO₄, and homogenized by a PT-10 Willem's Polytron tissue homogenizer (Kinematik GMBH, Luzem, Switzerland) at 0°C. The tube containing the tissue was removed, and a second tube containing 4 ml of 0.4N HClO₄ was used to rinse the homogenizer. This solution was added to the first tube containing the tissue. This procedure was then repeated using 2 ml of 0.4N HClO₄, which was also used to rinse the second tube. Therefore, the total volume was 10 ml plus the volume obtained from the tissue. The tube containing the homogenized tissue was centrifuged at approximately 34,800 g for 15 minutes, and the super-
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natant fluid was removed. A 9-ml sample of the supernatant fluid was pipetted into a 50-ml beaker, and distilled water was added to make the total volume up to 10 ml. Then, 1 ml of 1-M Tris buffer (pH 8.4), 500 μl of 1% ethylenediaminetetraacetic acid (EDTA), and 100 μl of Na₂SO₄ were added to prevent auto-oxidation. The pH of this solution was rapidly adjusted to 8.4 with 5.0N NaOH; 0.4M HClO₄ was used to back-titrage when necessary. Catecholamines were absorbed on alumina and eluted with 0.3M acetic acid; 1-ml samples of the eluate were oxidized to adrenochrome with 0.01% (w/v) K₃FeCN₅ buffered with 1.5M sodium acetate. Rearrange-ment to the fluorescent adrenolutine was carried out with 2.5M NaOH. A 1% (v/v) solution of thioglycolic acid and a 0.3% (w/v) solution of ascorbic acid were used to stabilize the adrenochrome.

All steps after column elution were automated by coupling parts of a technical autoanalyzer (Technicon Corp.) to a spectrofluorometer (American Instrument Co.) via a specially designed flow cell. Blanks were determined on separate samples by introducing sample flow after the points at which the stabilizing reagent entered the flow system. External standards in 0.3M acetic acid were used for all determinations. Recovery of norepinephrine averaged 75% (range 69 to 110%).

DRUGS

The concentration of norepinephrine bitartrate (Winthrop) is expressed as the free base in micrograms. The concentrations of all other drugs are expressed as the free acid or the free base in micrograms/liter: pyruvic acid and dl-lactic acid were used as the sodium salts, succinic acid was used as the sodium hexahydrate, dl-2-glycerophosphate, sorbitol (d-glucitol), galactitol (dulcitol), L-amino-n-butyric acid, l-glutamic acid, l-aspartic acid, adenosine-3',5'-cyclic monophosphate, and guanosine-3',5'-cyclic monophosphate were used as the free acids, adenosine-5'-triphosphate and adenosine-5'-diphosphate were used as the disodium salts, adenosine-3',5'-diphosphate was used as the dithium salt, and N₂O₂-dibutyryl-adenosine-3',5'-monophosphate was used as the sodium salt. All were obtained from Sigma. Adenosine, inosine, 2-deoxy-d-glucose, β-D-fructose, D-mannose, D-galactose, D-xylene, D-mannitol, and sucrose were also obtained from Sigma. Iodoacetic acid was obtained from Aldrich, prostaglandins E₁, E₂, A₁, and F₂, from Upjohn, atropine sulfate from K & K Laboratories, and propranolol from Ayerst.

STATISTICAL ANALYSIS

Paired and unpaired t-tests and analysis of variance were performed according to the methods described by Steel and Torrie (13).

Results

In mesenteric arteries perfused with Tyrode's solution at 35°C, nerve stimulation, 6 Hz (pulses 1

msec in duration) at 4-minute intervals, produced nondecremental vasoconstrictor responses over a period of 1–1.5 hours. The responses then declined gradually over the next 3 hours. The vasoconstrictor response to nerve stimulation was markedly potentiated when glucose was removed from the perfusion medium (Fig. 1a). Potentiation became maximum after 60–90 minutes, remained at this level for about 1 hour, and then declined gradually over the next 2 hours. Removal of glucose did not alter the basal perfusion pressure (Fig. 1a). Figure 1b illustrates that 5.6 mM glucose (the concentration normally present in Tyrode's solution) abolished the potentiated vasoconstrictor response to nerve stimulation produced by glucose deprivation.

To determine whether augmentation of the vasocostrictor response to nerve stimulation after glucose deprivation resulted from increased vascular reactivity to norepinephrine released from nerve fibers, inhibition of norepinephrine uptake, or increased release of the adrenergic transmitter, we injected enough norepinephrine (0.5–3 μg) to produce a vasoconstrictor response equal to that obtained with nerve stimulation (Fig. 1c). Removal of glucose from the perfusion fluid resulted in a slight augmentation of the equiconstrictor response to injected norepinephrine, but this potentiated response was considerably less than the potentiated response to nerve stimulation. The augmentation of the vasoconstrictor response to injected norepinephrine produced by glucose deprivation was also abolished by addition of glucose and restored by subsequent removal of glucose from the perfusion fluid.

We then determined the ability of various concentrations of glucose to reduce the maximally potentiated vasoconstrictor response to nerve stimulation and the equiconstrictor response to injected norepinephrine produced in the absence of glucose (Fig. 2). Infusion of glucose at concentrations lower than 5.6 × 10⁻³ mM did not affect the potentiated vasoconstrictor responses. Glucose in concentrations of 5.6 × 10⁻³ mM significantly (P < 0.001) reduced the maximally potentiated vasoconstrictor response to nerve stimulation but did not alter the response to injected norepinephrine (Fig. 2). However, higher concentrations of glucose did reduce the equiconstrictor response to injected norepinephrine. Addition of glucose in successively higher concentrations always reduced the maximally potentiated vasoconstrictor response to nerve stimulation more than it reduced the response to injected norepinephrine (P < 0.001) (Fig. 2). Infusion of glucose in concentrations up to 56 mM did not alter
Effects of glucose (Gluc) and glucose deprivation on the vasoconstrictor responses of perfused rat mesenteric arteries to postganglionic sympathetic nerve stimulation (NS) and to injected norepinephrine (NE). Mesenteric vessels were perfused with Tyrode's solution with or without glucose. Sympathetic nerves to the vessels were stimulated at 4-minute intervals. Norepinephrine (0.5 μg) was injected directly into the arterial cannula at 4-minute intervals.

a: Removal of glucose from the perfusion fluid potentiated the vasoconstrictor response to nerve stimulation.
b: The potentiated response to nerve stimulation was abolished by infusion of glucose, 5.6 mM for 20 minutes, but it recurred after about 80 minutes of perfusion with glucose-free Tyrode's solution.

c: Removal of glucose from the perfusion fluid produced only a small potentiation in the vasoconstrictor response to injected norepinephrine; this potentiation was abolished by infusion of 5.6 mM glucose but recurred within 24 minutes after glucose deprivation had been reinstituted.
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the basal pressure. However, higher concentrations constricted the mesenteric vessels, resulting in an increase in the perfusion pressure.

EFFECTS OF GLUCOSE DEPRIVATION ON THE NOREpinePHRINE CONTENT OF THE MESENTERIC ARTERIES DURING SYMPATHETIC NERVE STIMULATION

To determine if the potentiation of the vasoconstrictor response to sympathetic nerve stimulation produced by glucose deprivation was due to depletion of norepinephrine from its storage sites, we measured the norepinephrine content of mesenteric arteries after stimulation of their sympathetic fibers at 4-minute intervals for 90 minutes in the absence and the presence of glucose. The norepinephrine content of mesenteric arteries perfused in the presence of glucose, 0.291 ± 0.032 (SE) μg/g, was 28% greater than the norepinephrine content of vessels perfused in the absence of glucose, 0.209 ± 0.032 μg/g. However, this difference was not significant (0.5 > P < 0.1).

EFFECTS OF COCAINE ON THE VASOCONSTRICTOR RESPONSES TO SYMPATHETIC NERVE STIMULATION AND TO INJECTED NOREPINEPHRINE IN THE PRESENCE AND THE ABSENCE OF GLUCOSE

Since the equiconstrictor response to norepinephrine was much less affected by glucose deprivation than was the vasoconstrictor response to nerve stimulation, altered neuronal reuptake of norepinephrine was probably not a major factor in the potentiation of the vasoconstrictor response to nerve stimulation. To test this interpretation, we examined the effect of cocaine (14) on the equiconstrictor responses to nerve stimulation and to injected norepinephrine in the presence and the absence of glucose. In the presence of glucose, cocaine (1 μg/ml) potentiated the vasoconstrictor response to nerve stimulation by 166 ± 26% (SE) and that to injected norepinephrine by 126 ± 18% (9 experiments) without altering the basal pressure. The maximally potentiated vasoconstrictor responses to nerve stimulation (14 experiments)

![Figure 2](http://circres.ahajournals.org/)

**Figure 2**

Effects of various concentrations of glucose on the equiconstrictor responses of perfused rat mesenteric arteries to nerve stimulation and to injected norepinephrine after the response has been maximally augmented by glucose deprivation. The control responses, taken as 100%, represent the maximal vasoconstrictor responses to nerve stimulation and to injected norepinephrine (0.5-3 μg) in the absence of glucose. Infusion of glucose for 16-20 minutes reduced the responses to these adrenergic stimuli. The number of experiments is given in the circle at the bottom of each column. The number on the top left of each column represents the percent reduction in the response to adrenergic stimuli. An asterisk on the top of a column denotes a significant reduction compared with the control response. For each concentration greater than 5.6 x 10^-3 M, glucose inhibited the vasoconstrictor response to nerve stimulation more than it inhibited the response to injected norepinephrine (significant differences are indicated by a bracket and an asterisk at the top of the paired columns). Vertical brackets represent the SE.

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and to injected norepinephrine (12 experiments) in the absence of glucose were also further potentiated by the infusion of cocaine (1 μg/ml) by 163 ± 23% and 126 ± 18%, respectively; the degree of augmentation was similar to that produced in the presence of glucose. Thus, facilitation of adrenergic transmission by glucose deprivation was independent of a major effect on the adrenergic neuron's amine uptake mechanism.

**Effects of Glucose Metabolites on the Vasoconstrictor Responses of Mesenteric Arteries to Sympathetic Nerve Stimulation and to Injected Norepinephrine in the Absence and the Presence of Glucose**

If the greater augmentation of the vasoconstrictor response to nerve stimulation compared with the response to injected norepinephrine produced by glucose deprivation is due to enhanced release of the adrenergic transmitter, then it may result from depletion of glucose metabolites formed in the sympathetic nerves. Glucose metabolites could serve as an energy source controlling the release of the adrenergic transmitter, exert a direct inhibitory action on the release process, or both. To explore this possibility, we examined the effects of glucose metabolites on the equiconstrictor responses of perfused rat mesenteric arteries to postganglionic sympathetic nerve stimulation and to injected norepinephrine in the absence and the presence of glucose. We also studied the inhibitory effect of glucose on the potentiated vasoconstrictor response to nerve stimulation and the modification of this effect by inhibitors of glucose metabolism.

**Effects of Pyruvic, Lactic, and Succinic Acids and Glycerophosphate.**—Infusion of either pyruvic or lactic acid at concentrations less than 1.1 × 10⁻² mM did not alter the maximally potentiated vasoconstrictor responses of mesenteric arteries to nerve stimulation or to injected norepinephrine. At concentrations of 1.1 × 10⁻² mM, pyruvic and lactic acids reduced the potentiated vasoconstrictor responses to nerve stimulation but not those to injected norepinephrine (Fig. 3). At concentrations of 1.1 × 10⁻¹ mM, pyruvic and lactic acids produced a greater reduction in the potentiated response to nerve stimulation, but only pyruvic acid reduced the vasoconstrictor response to injected norepinephrine. In 15 experiments, the same concentrations of pyruvic and lactic acids failed to alter the vasoconstrictor responses to nerve stimulation and equiconstrictor responses to injected norepinephrine in the presence of glucose. Neither succinic acid (8 experiments) nor glycerophosphate (6 experiments) in concentrations as high as 8.5 mM and 9.2 mM, respectively, affected the vasoconstrictor responses to nerve stimulation or the equiconstrictor responses to injected norepinephrine either in the absence or the presence of glucose. Basal pressure was not changed by pyruvic, lactic, or succinic acids or by glycerophosphate.

**Effects of Sugar Alcohols.**—Sugar alcohols, such as sorbitol, formed from glucose have been identified in rat peripheral nerves and brain (15). To determine if the inhibitory effect of glucose on adrenergic transmission is dependent on the formation of sugar alcohols in sympathetic nerves, we examined the effects of two sugar alcohols, sorbitol and galactitol, on the vasoconstrictor responses of rat mesenteric arteries to nerve stimulation and to injected norepinephrine. Infusions of either sorbitol (five experiments) or galactitol (four experiments) at rates which produced concentrations as high as 5.6 mM did not alter the basal perfusion pressure, the responses to nerve stimulation, or the responses to injected norepinephrine in the absence or the presence of glucose.

**Effects of Amino Acids, γ-Aminobutyric, Glutamic, and Aspartic Acids, and Glycine.**—Some amino acids derived from glucose (16) have been proposed to be inhibitory neurotransmitters at certain sites in the central nervous system (17). To determine if glucose inhibits release of the adrenergic transmitter through the formation of these agents in sympathetic nerve fibers, we examined their effects on the vasoconstrictor responses to nerve stimulation and to injected norepinephrine. In the absence of glucose, the basal pressure and the maximally potentiated vasoconstrictor responses to nerve stimulation and to injected norepinephrine remained unaltered during the infusion of either γ-aminobutyric acid (five experiments) or glutamic acid (six experiments) at rates effecting concentrations as high as 10 mM. These agents were also ineffective in the presence of glucose. Aspartic acid, in concentrations of 7.5 × 10⁻¹ and 7.5 mM, did not affect the basal perfusion pressure; in the absence of glucose, it did reduce the potentiated vasoconstrictor response to sympathetic nerve stimulation but not that to injected norepinephrine (Fig. 3). In the presence of glucose, aspartic acid did not affect the vasoconstrictor responses to either adrenergic stimulus. Glycine, at concentrations greater than 1.33 mM, did not affect the basal pressure but did similarly reduce the potentiated equiconstrictor responses to nerve stimulation and to injected norepinephrine in the absence of glucose (Fig. 3). Glycine was without effect in the presence of glucose.
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RESPONSE TO NERVE STIMULATION  
RESPONSE TO INJECTED NOREPINEPHRINE

\[ \Delta = P < 0.001 \]
\[ \Delta = P < 0.01 \]
\[ \Delta = P < 0.025 \]
ns = not significant

FIGURE 3
Effects of pyruvate, lactate, aspartate, and glycine on the vasoconstrictor response of mesenteric arteries to sympathetic nerve stimulation and an equiconstrictor response to injected norepinephrine. The control responses taken as 100\%, represent the maximal vasoconstrictor responses to nerve stimulation and to injected norepinephrine in the absence of glucose. The number of experiments is given in the circle at the bottom of each column. The number on the top left of each column represents the percent reduction in the response to adrenergic stimuli. An asterisk or a triangle at the top of a column denotes a significant reduction compared with the control response. Significant differences in the degree of reduction in the response to nerve stimulation compared with the degree of reduction in the response to injected norepinephrine are indicated above the bracket at the top of the paired columns. Vertical brackets represent the SE.

EFFECTS OF PURINE NUCLEOTIDES ON THE VASOCONSTRICTOR RESPONSES OF MESENTERIC ARTERIES TO SYMPATHETIC NERVE STIMULATION AND TO INJECTED NOREPINEPHRINE IN THE ABSENCE AND THE PRESENCE OF GLUCOSE

Since purine nucleotides derived from glucose are the major source of energy in various tissues, enhanced release of the adrenergic transmitter produced by glucose deprivation may result from diminished amounts of energy supplied by nucleotides for control of the release process. Alternatively, nucleotides may directly inhibit the release mechanism. Despite the fact that purine nucleotides do not easily cross the cell membrane (18), we examined their possible involvement in the inhibitory action of glucose on adrenergic transmission in view of their presence in plasma (19) and their release from nerves (18).

Adenosine-5-triphosphate (ATP), adenosine-5'-diphosphate (5'-ADP), adenosine-3',5'-diphosphate (3',5'-ADP), adenosine-5'-monophosphate (5'-AMP), adenosine-3',5'-cyclic monophosphate (3',5'-cAMP), N^6,O^2-dibutyryl-adenosine-3',5'-cyclic monophosphate (dibutyryl-3',5'-cAMP), guanosine-3',5'-cyclic monophosphate (3',5'-cGMP), adenosine, and inosine in concentrations as high as \( 4 \times 10^{-4} \) mM did not affect basal perfusion pressure. Infusion of ATP at rates which established concentrations of \( 1.8 \times 10^{-4} \) mM reduced the vasoconstrictor response to nerve stimulation but did not affect an equiconstrictor response to injected norepinephrine (Fig. 4). Ten times higher concentrations of ATP reduced the vasoconstrictor responses to nerve stimulation and
to injected norepinephrine, but the former was reduced to a greater degree than the latter (Fig. 4). At concentrations of $2 \times 10^{-6}$ mM or greater, 5'-ADP decreased the maximally potentiated vasoconstrictor response to sympathetic nerve stimulation and an equiconstrictor response to injected norepinephrine ($P < 0.001$). The former was reduced more than the latter at concentrations of $2 \times 10^{-6}$ mM or $2 \times 10^{-4}$ mM, whereas, at a concentration of $2 \times 10^{-2}$ mM, 5'-ADP reduced the potentiated vasoconstrictor responses to nerve stimulation and to injected norepinephrine equally (Fig. 4). Concentrations of 3',5'-ADP and 5'-AMP of $2 \times 10^{-3}$ mM and $2.7 \times 10^{-3}$ mM, respectively, reduced the maximally potentiated response to nerve stimulation and an equiconstrictor response to injected norepinephrine in the absence of glucose (Fig. 4); lower concentrations were without effect. Adenosine in concentrations of $3.7 \times 10^{-3}$ mM or greater affected the equiconstrictor responses to nerve stimulation and to injected norepinephrine in a similar degree (Fig. 4). The reduction in the vasoconstrictor response to nerve stimulation produced by nucleotides in the absence of glucose was usually greater than the reduction in an equiconstrictor response to injected norepinephrine. Analysis of variance revealed that ATP reduced the maximally potentiated vasoconstrictor response to nerve stimulation to a greater degree ($P < 0.01$) than it reduced an equiconstrictor response to injected norepinephrine compared with the other nucleotides at a similar concentration in the absence of glucose (Fig. 4).

Infusion of 3',5'-cAMP (six experiments), dibu-
tyryl-3',5'-cAMP (four experiments), and 3',5'-cGMP (five experiments) at rates which produced concentrations of $2.6 \times 10^{-3}$ mM did not affect the vasoconstrictor responses to adrenergic stimuli either in the absence or the presence of glucose. Inosine (seven experiments) in concentrations as high as $3.7 \times 10^{-3}$ mM did not alter the responses to injected norepinephrine and to nerve stimulation.

The inhibitory effect of nucleotides on adrenergic transmission in rat mesenteric arteries is independent of the concentration of glucose; the nucleotides modified the vasoconstrictor responses to either adrenergic stimulus similarly in the presence and the absence of glucose. In four experiments with ATP ($1.8 \times 10^{-2}$ mM) in the presence of glucose, the degree of reduction in the vasoconstrictor response to nerve stimulation was not different from that observed in the absence of glucose.

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**Modification by Inhibitors of Glucose Metabolism.**

**2-Deoxy-D-Glucose and Iodoacetic Acid.** From the Effects on Responses of Mesenteric Arteries to Sympathetic Nerve Stimulation and to Injected Norepinephrine at Different Temperatures

To determine if a glucose metabolite is involved in the inhibitory effect of glucose on release of the adrenergic transmitter, we studied the effects of 2-deoxy-D-glucose (2-DG) and iodoacetic acid (IAA), inhibitors of glucose metabolism, on the mesenteric vasoconstrictor responses to adrenergic stimuli as modified by glucose. 2-DG prevents utilization of glucose by competitive antagonism of the phosphohexose isomerase reaction (20), and IAA inhibits glycolysis and oxidative metabolism of pyruvate (21).

**Effects of 2-Deoxy-D-Glucose.**—In seven experiments, the maximally potentiated vasoconstrictor response of mesenteric arteries to nerve stimulation in the absence of glucose was transiently augmented by $3.3 \times 10^{-1}$ mM 2-DG (Fig. 5Aa). The equiconstrictor response to injected norepinephrine in the presence or the absence of glucose remained unaltered or showed a slight reduction (five experiments). (Fig. 5Ab). The maximally potentiated vasoconstrictor response to nerve stimulation in the absence of glucose was reduced by an infusion of glucose (Fig. 5Ac). However, the simultaneous infusion of $3.3 \times 10^{-1}$ mM 2-DG restored the potentiated vasoconstrictor response despite continued infusion of glucose. Concentrations of 2-DG five to ten times higher than the concentrations of glucose were required to abolish the inhibitory effect of glucose on the vasoconstrictor response to nerve stimulation. The maximally potentiated vasoconstrictor response of mesenteric vessels to nerve stimulation produced in the absence of glucose was inhibited by $5.6 \times 10^{-2}$ mM glucose and then restored by the simultaneous infusion of 2-DG at rates which yielded a concentration of $6.6 \times 10^{-1}$ mM. Lesser concentrations of 2-DG were without any effect on the inhibitory action of $5.6 \times 10^{-2}$ mM glucose, whereas higher concentrations of 2-DG produced a reduction of the vasoconstrictor response to injected norepinephrine.

As we have indicated, pyruvic acid in concentrations of $1.1 \times 10^{-2}$ or $1.1 \times 10^{-1}$ mM reduces the maximally potentiated vasoconstrictor response to nerve stimulation induced by glucose deprivation. Simultaneous infusion of 2-DG at rates which yielded concentrations of $3.3 \times 10^{-1}$ or $6.6 \times 10^{-1}$ mM failed to abolish the inhibitory effect of pyruvic acid on the vasoconstrictor response to nerve stimulation. We have indicated, pyruvic acid in concentrations of $1.1 \times 10^{-2}$ or $1.1 \times 10^{-1}$ mM reduces the maximally potentiated vasoconstrictor response to nerve stimulation induced by glucose deprivation. Simultaneous infusion of 2-DG at rates which yielded concentrations of $3.3 \times 10^{-1}$ or $6.6 \times 10^{-1}$ mM failed to abolish the inhibitory effect of pyruvic acid on the vasoconstrictor response to nerve stimulation. The inhibitory effect of nucleotides on adrenergic release of the transmitter, we studied the effects of 2-deoxy-D-glucose (2-DG) and iodoacetic acid (IAA), inhibitors of glucose metabolism, on the mesenteric vasoconstrictor responses to adrenergic stimuli as modified by glucose. 2-DG prevents utilization of glucose by competitive antagonism of the phosphohexose isomerase reaction (20), and IAA inhibits glycolysis and oxidative metabolism of pyruvate (21).

**Effects of Iodoacetic Acid.**—Infusion of iodoacetic acid (IAA) at rates which established concentrations of $5.4 \times 10^{-2}$ mM did not alter the maximal vasoconstrictor responses of mesenteric arteries to either sympathetic nerve stimulation (13 experiments) or injected norepinephrine (10 experiments) produced by glucose deprivation (Fig. 5Ba, 5Bb). Since higher concentrations of IAA ($5.4 \times 10^{-1}$ mM) augmented the vasoconstrictor responses to adrenergic stimuli, we used the lower concentration to determine the ability of IAA to restore the potentiated vasoconstrictor response to nerve stimulation after its inhibition by glucose. The potentiated vasoconstrictor response to nerve stimulation produced by glucose deprivation was inhibited by infusion of glucose but was, in turn, restored by the simultaneous infusion of $5.4 \times 10^{-2}$ mM IAA despite the continued infusion of glucose (Fig. 5Bc). This effect of IAA was observed in all 7 of the experiments in which it was tested.

In contrast to 2-DG, IAA reversed the inhibitory action of $1.1 \times 10^{-1}$ mM pyruvic acid on the potentiated vasoconstrictor response to nerve stimulation. However, the potentiated vasoconstrictor response could be reduced again by increasing the concentration of pyruvic acid to $1.1$ mM by adding $1.1 \times 10^{-1}$ mM lactic acid to the perfusion medium.

**Effects of Lowering Temperature (22°C).**—To determine whether glucose and its metabolite, pyruvate, inhibited adrenergic transmission facilitated by glucose deprivation by functioning as an energy source or by a direct effect, we also studied the inhibitory effects of glucose and pyruvic acid at 22°C and the modification of their action by 2-DG and IAA. At 22°C, metabolism of glucose and
Effect of 2-deoxy-D-glucose (2-D Gluc), $3.3 \times 10^{-1}$ mM (A), and iodoacetic acid (IAA), $5.4 \times 10^{-2}$ mM (B), on the vasoconstrictor responses of rat mesenteric arteries to sympathetic nerve stimulation (NS) (a and c) and to injected norepinephrine (NE) (b). Sympathetic nerves were stimulated at 4-minute intervals. Norepinephrine (0.8 μg) was injected directly into the arterial cannula at 4-minute intervals. A: During 36 minutes of infusion, 2-deoxy-D-glucose produced a transient increase in the vasoconstrictor response to nerve stimulation (a) previously augmented by glucose deprivation. The vasoconstrictor response to injected norepinephrine obtained in the absence of glucose was slightly reduced by the infusion of 2-deoxy-D-glucose for 24 minutes (b). The reduction by $5.6 \times 10^{-3}$ mM glucose of the vasoconstrictor response to nerve stimulation augmented by glucose deprivation was abolished by the simultaneous infusion of 2-deoxy-D-glucose (c). B: The vasoconstrictor response to nerve stimulation maximally augmented by glucose deprivation was further slightly potentiated by an infusion of iodoacetic acid (a), whereas the response to injected norepinephrine (b) was not altered. However, the simultaneous infusion of iodoacetic acid abolished the reduction by 5.6 mM glucose of the vasoconstrictor response to nerve stimulation previously potentiated by glucose deprivation (c).

Pyruvate is presumably reduced; therefore, their inhibitory effect on the potentiated vasoconstrictor response to nerve stimulation should be diminished if it depends on their ability to provide energy to the mechanism which regulates release of the neurotransmitter. This diminution was not found: the vasoconstrictor response of rat mesenteric arteries to sympathetic nerve stimulation was augmented two- to fourfold, whereas the response to injected norepinephrine was reduced to between a half and a third of the control response when the temperature of the perfusion fluid was decreased from 35°C to 22°C (22). Removal of glucose from the fluid perfusing mesenteric vessels at 22°C caused further augmentation of the vasoconstrictor response to nerve stimulation in a way similar to that seen at 35°C. However, at 22°C as at 35°C, addition of glucose and pyruvic acid inhibited the potentiated vasoconstrictor response to nerve stimulation in ten experiments by 81 ± 5% (SE) and 74 ± 8%, respectively, and to injected norepinephrine by 10 ± 2% and 3 ± 2%, respectively. Moreover, at 22°C, the maximally potentiated vasoconstrictor response to nerve stimulation in the absence of glucose was reduced by the infusion of $5.6 \times 10^{-3}$ mM glucose but was restored by the simultaneous infusion of either $3.3 \times 10^{-3}$ mM 2-DG (five experiments) or $2.7 \times 10^{-2}$ mM IAA (six experiments).
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EFFECTS OF PROSTAGLANDINS AND INHIBITORS OF PROSTAGLANDIN SYNTHESIS ON THE VASOCONSTRICTOR RESPONSES OF MESENTERIC ARTERIES TO SYMPATHETIC NERVE STIMULATION AND TO INJECTED NOREPINEPHRINE IN THE ABSENCE AND THE PRESENCE OF GLUCOSE

Prostaglandins of the E (PGE) series have been proposed to modulate the release of norepinephrine from adrenergic nerve endings (23). We examined the actions of the major prostaglandins and two inhibitors of prostaglandin synthesis, indomethacin and meclofenamate (24), on the vasoconstrictor responses to adrenergic stimuli to determine if the inhibitory effect of glucose and its metabolites on the release of the adrenergic transmitter is the result of increased synthesis of prostaglandins.

Prostaglandins in concentrations greater than 2.8 x 10^{-4} \text{ mM} augmented further the potentiated vasoconstrictor response to nerve stimulation and to injected norepinephrine produced by glucose deprivation without affecting basal perfusion pressure; PGE_{1a}, PGE_{2a}, and PGA_{1} (1.4 x 10^{-6} \text{ mM}) and PGF_{2\alpha} (1.4 x 10^{-4} \text{ mM}) produced 50-100\% augmentation of the potentiated vasoconstrictor responses to both adrenergic stimuli (four to six experiments for each prostaglandin). This effect of prostaglandins was also demonstrated in the presence of glucose. Infusion of either indomethacin (five experiments) or meclofenamate (four experiments) at rates which produced concentrations of 2.9 x 10^{-4} \text{ mM} or higher reduced the vasoconstrictor responses to nerve stimulation and to injected norepinephrine in the presence or the absence of glucose. Although indomethacin and meclofenamate produced an overall reduction in the vasoconstrictor response to nerve stimulation to a similar degree in the presence experiments, in three experiments glucose deprivation still augmented the response to nerve stimulation.

EFFECTS OF AUTONOMIC BLOCKING AGENTS ON THE INHIBITORY EFFECT OF GLUCOSE AND PYRUVIC ACID ON THE POTENTIATED VASOCONSTRICTOR RESPONSES OF MESENTERIC ARTERIES TO SYMPATHETIC NERVE STIMULATION AND TO INJECTED NOREPINEPHRINE

To determine if inhibition by glucose of the potentiated vasoconstrictor responses to adrenergic stimuli produced by prior glucose deprivation is due to stimulation of muscarinic inhibitory pre- or postsynaptic receptors or adrenergic \( \beta \) receptors (9, 25-27), we investigated the effects of muscarinic and \( \beta \)-receptor blocking agents on the vasoconstrictor responses to adrenergic stimuli in the absence and the presence of glucose. We found that 1.4 x 10^{-4} \text{ mM} atropine and 1.9 x 10^{-4} \text{ mM} propranolol abolished the inhibitory effect of 2.75 x 10^{-4} \text{ mM} acetylcholine and 1.2 x 10^{-3} \text{ mM} isoproterenol, respectively, on the vasoconstrictor responses of mesenteric arteries to nerve stimulation and to injected norepinephrine (9, 27) but did not alter the potentiated response to nerve stimulation or to injected norepinephrine produced by glucose deprivation (four experiments). The inhibitory actions of glucose and pyruvic acid on the potentiated responses to adrenergic stimuli were not affected (six experiments) by these autonomic blocking agents.

EFFECTS OF ALTERATIONS IN THE CONCENTRATIONS OF DIVALENT AND MONOVALENT CATIONS ON THE VASOCONSTRICTOR RESPONSES OF MESENTERIC ARTERIES TO SYMPATHETIC NERVE STIMULATION AND TO INJECTED NOREPINEPHRINE IN THE PRESENCE AND THE ABSENCE OF GLUCOSE

Since release of the adrenergic transmitter is affected by changes in the ionic content of the extracellular fluid (28) and since the transport of some cations, notably Na\(^{+}\), has been shown to be associated with glucose transport (29), we examined the inhibitory action of glucose in terms of possible changes in the permeability of the neuronal membrane to Ca\(^{2+}\), Mg\(^{2+}\), Na\(^{+}\), and K\(^{+}\).

Effects of Changes in Calcium Chloride Concentration.—The concentration of CaCl\(_{2}\) in Tyrode's solution is 1.8 \text{ mM}. Raising the Ca\(^{2+}\) concentration of the perfusion medium from 1.8 to 5.4 \text{ mM} potentiated \((P < 0.001)\) the vasoconstrictor response to nerve stimulation to a similar degree in the presence and the absence of glucose (Fig. 6). In contrast, increasing the Ca\(^{2+}\) concentration reduced the equiconstrictor response to injected norepinephrine to a similar degree in the presence and the absence of glucose. Decreasing the concentration of Ca\(^{2+}\) from 1.8 to 0.9 \text{ mM} reduced the response to nerve stimulation in the presence and the absence of glucose; the response to injected norepinephrine was not altered. These changes in Ca\(^{2+}\) concentrations did not affect the basal perfusion pressure.

Effects of Changes in Magnesium Chloride Concentration.—The concentration of MgCl\(_{2}\) in Tyrode’s solution is 1.1 \text{ mM}. Raising the concentration of Mg\(^{2+}\) from 1.1 to 4.4 \text{ mM} both in the presence and the absence of glucose reduced the equiconstrictor responses to nerve stimulation and to injected norepinephrine, the former being more affected than the latter (Fig. 6). Lowering the Mg\(^{2+}\) concentration from 1.1 to 0.3 \text{ mM} did not affect the equiconstrictor responses to adrenergic stimuli (three experiments).

Effects of Changes in Sodium Chloride Concentra-
Effects of increased CaCl₂ and MgCl₂ concentration on the equiconstrictor responses of rat mesenteric arteries to sympathetic nerve stimulation and to injected norepinephrine in the presence and the absence of glucose. The control responses, taken as 100%, represent the vasoconstrictor responses to nerve stimulation and to injected norepinephrine (0.5-3 μg) of mesenteric vessels perfused with glucose-containing or glucose-free Tyrode's solution. CaCl₂ and MgCl₂ were added to the perfusion fluid to increase their concentration. The number at the top left of each column indicates the percent increase (+) or decrease (-) in the responses to nerve stimulation and to injected norepinephrine. An asterisk at the top of a column shows the significant increase or decrease in the responses to adrenergic stimuli compared with the control. Significant differences in the degree of augmentation or reduction in the responses to nerve stimulation and to injected norepinephrine produced by increased CaCl₂ and MgCl₂ in the presence of glucose compared with that in the absence of glucose are indicated by brackets with asterisks at the top of paired columns. Other notation is the same as it is in Figures 3 and 4.

- The concentration of NaCl in Tyrode's solution is 137 mM. Concentrations of Na⁺ greater than 200 mM markedly constricted the mesenteric blood vessels, resulting in an increase in basal perfusion pressure. In the presence of glucose, increasing the concentration of Na⁺ from 137 to 164 mM did not affect the response to nerve stimulation (Fig. 7) in a sustained manner, although a transient decrease in the initial response was observed (Fig. 8). In contrast, during glucose deprivation, raising the Na⁺ concentration augmented the potentiated vasoconstrictor response to nerve stimulation after an initial brief reduction. The equiconstrictor response to injected norepinephrine was reduced to the same degree in the presence and the absence of glucose when the Na⁺ concentration was increased (Fig. 7).

- Lowering the Na⁺ concentration from 137 to 110 mM did not affect the basal perfusion pressure, variably affected the vasoconstrictor response to nerve stimulation (ten experiments), and markedly potentiated the response to injected norepinephrine.

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Effects of Increased NaCl and KCl Concentration on the Equiconstrictor Responses of Rat Mesenteric Arteries to Sympathetic Nerve Stimulation and to Injected Norepinephrine in the Absence and the Presence of Glucose (5.6 mM). The control responses, taken as 100%, represent the vasoconstrictor responses to nerve stimulation and to injected norepinephrine of mesenteric vessels perfused with glucose-free or glucose-containing Tyrode's solution. Concentrations of NaCl and KCl were increased by adding them to the perfusion fluid. See the legends to Figures 3, 4, and 5 for further explanation.

Effects of Changes in Potassium Chloride Concentration. —Increasing the concentration of KCl in Tyrode's solution fourfold, i.e., from 2.7 mM to 10.8 mM, augmented the vasoconstrictor response to nerve stimulation in the presence of glucose, whereas in the absence of glucose the potentiated vasoconstrictor response to nerve stimulation was reduced (Fig. 7). In contrast, the response to injected norepinephrine was not significantly affected by raising the K+ concentration in the presence of glucose but was increased in its absence. Lowering K+ concentration from 2.7 to 0.7 mM or removal of K+ from the perfusion fluid augmented the responses to nerve stimulation and to injected norepinephrine and increased the basal perfusion pressure both in the absence and the presence of glucose (seven experiments).

EFFECTS OF CHANGES IN OSMOLARITY OF THE PERFUSION FLUID ON THE VASOCONSTRICTOR RESPONSES OF MESENTERIC ARTERIES TO SYMPATHETIC NERVE STIMULATION AND TO INJECTED NOREPINEPHRINE

The augmentation of the vasoconstrictor responses to adrenergic stimuli produced by glucose deprivation (5.6 mM) may have resulted from the decreased osmolarity of the perfusion fluid. Similar results were obtained when Na+ was replaced with equimolar amounts of sucrose (27 mM). Greater reductions in Na+ concentration (>37 mM) either alone or after replacement with equimolar sucrose produced an increase in the basal perfusion pressure.
larly, changes in the responses to either nerve stimulation or injected norepinephrine produced by increased Ca$^{2+}$, Mg$^{2+}$, Na$^{+}$, or K$^{+}$ may have resulted from increased osmolarity of the perfusion medium. Therefore, we studied the effects of inert sugars, i.e., mannitol and sucrose, substituted in equimolar amounts for glucose (5.6 mM) after removal of the latter and their effects at a high concentration, namely, after the addition of 27 mM sugar to normal Tyrode's solution.

Perfusion of mesenteric vessels with Tyrode's solution in which the glucose had been replaced by equimolar amounts of either mannitol or sucrose (5.6 mM) did not affect the potentiated vasoconstrictor response to nerve stimulation (six experiments). Furthermore, the simultaneous infusion of glucose (5.6 mM) reduced the potentiated vasoconstrictor response to nerve stimulation; the potentiated response was restored by perfusion with glucose-free Tyrode's solution containing either mannitol or sucrose (5.6 mM). Infusion of mannitol or sucrose (5.6 mM) did not alter the basal perfusion pressure or the vasoconstrictor response to injected norepinephrine.

Infusion of mannitol (27 mM) reduced the vasoconstrictor response of mesenteric arteries to nerve stimulation and to injected norepinephrine similarly in the presence and the absence of glucose (Fig. 9). The reduction in the vasoconstrictor response to injected norepinephrine was greater than that to nerve stimulation both in the presence and the absence of glucose. Sucrose (27 mM) reduced the vasoconstrictor response to injected norepinephrine similarly in the presence and the absence of glucose, whereas the response to nerve stimulation was reduced only in the absence of glucose. Thus, mannitol and sucrose reduced the vasoconstrictor response to injected norepinephrine more than they reduced the response to nerve stimulation either in the absence or the presence of glucose (Fig. 9).

**EFFECTS OF OTHER SUGARS ON THE POTENTIATED VASOCONSTRICTOR RESPONSES OF MESENTERIC ARTERIES TO SYMPATHETIC NERVE STIMULATION AND TO INJECTED NOREpinePHRINE**

Fructose, mannose, galactose, and xylose reduced the potentiated vasoconstrictor response to sympathetic nerve stimulation produced by glucose deprivation more than they reduced the potentiated vasoconstrictor response to injected norepinephrine (Fig. 10). We applied Duncan's multiple range test to determine differences among these sugars in terms of their effects on adrenergic stimuli. Fructose and mannose were as effective as glucose but significantly more effective ($P < 0.5$) than galactose and xylose (glucose = fructose = mannose > xylose = galactose) in reducing the potentiated vasoconstrictor response to sympathetic nerve stimulation.

![Effects of increased NaCl concentration on the vasoconstrictor response of rat mesenteric arteries to sympathetic nerve stimulation in the presence (A) (5.6 mM) and the absence of glucose (Gluc) (B). Sympathetic nerves were stimulated at 6 Hz at 4-minute intervals. The concentration of Na$^{+}$ was increased by adding NaCl to the perfusion fluid. When the concentration of NaCl was increased from 137 to 164 mM, there was an initial reduction followed by return of the response to nerve stimulation to control levels in the presence of glucose (A) and an increase above control levels in the absence of glucose (B). When the NaCl concentration was lowered from 164 to 137 mM, the response to nerve stimulation was again initially reduced followed by an increase of the response to control levels.](http://circres.ahajournals.org/)

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Control response

Response to nerve stimulation

Response to injected norepinephrine

ns = not significant  Δ = P < .01  * = P < .001

Effects of mannitol (27 mM) and sucrose (27 mM) infused for 16–24 minutes on the equiconstrictor responses to sympathetic nerve stimulation and to injected norepinephrine of rat mesenteric arteries perfused with glucose-containing or glucose-free Tyrode's solution. Sympathetic nerves were stimulated at 4-minute intervals. Norepinephrine (0.5–3 μg) was injected into the arterial cannula. The control responses, taken as 100%, represent the responses to nerve stimulation and to injected norepinephrine obtained in the presence or the absence of glucose. Mannitol or sucrose was added to the perfusion fluid. Significant differences in the degree of reduction produced by mannitol or sucrose in the response to nerve stimulation compared with the response to injected norepinephrine in the presence and the absence of glucose are indicated by the brackets with an asterisk or triangles at the top of paired columns. Sucrose significantly (indicated by an asterisk at top of the column) reduced the response to nerve stimulation augmented by glucose deprivation.

Discussion

Glucose deprivation markedly potentiated the vasoconstrictor response of perfused rat mesenteric arteries to sympathetic nerve stimulation. The potentiated vasoconstrictor response to nerve stimulation was reduced when glucose was restored to the perfusion medium in a concentration one-thousandth that normally present in Tyrode's solution. Thus, glucose must inhibit the release of norepinephrine or affect the reactivity of vascular smooth muscle to norepinephrine released from nerves. Additional experiments established that the increased vasoconstrictor response to nerve stimulation was not primarily due to increased vascular reactivity to norepinephrine, inhibition of norepinephrine reuptake after its release from nerve terminals, or both, since the response to injected norepinephrine was augmented much less than the response to sympathetic nerve stimulation. More-
over, an inhibitor of neuronal uptake, cocaine (14), further augmented the already maximally potentiated equiconstrictor responses to nerve stimulation and to injected norepinephrine in the absence of glucose, thereby demonstrating that potentiation was independent of an effect on neuronal reuptake of norepinephrine. In addition, the potentiated vasoconstrictor response to nerve stimulation was significantly reduced by concentrations of glucose that did not alter the potentiated response to injected norepinephrine. Higher concentrations of glucose did reduce the maximal vasoconstrictor response to injected norepinephrine produced by removal of glucose, although always to a lesser degree than they reduced the response to nerve stimulation (Fig. 2). These differences were not the result of changes in vascular smooth muscle tone, since basal perfusion pressure, an index of vascular tone, was unaffected by removal of glucose. Thus, the potentiation of the response to nerve stimulation produced by glucose deprivation is most probably due to increased release of the adrenergic transmitter.

Enhanced release of the adrenergic transmitter from sympathetic nerve fibers could result from (1) rapid depletion of norepinephrine from its storage sites, e.g., as a result of a decrease in ATP to which norepinephrine is complexed (30), (2) a direct effect of glucose or a metabolite(s) on the adrenergic transmitter release mechanism, or (3) increased synthesis of norepinephrine. The first possibility was considered to be the least likely, since the norepinephrine content of mesenteric blood vessels after sympathetic nerve stimulation for 1.5 hours was similar in the presence and the absence of glucose. In fact, the norepinephrine content of mesenteric blood vessels determined after stimulation of their sympathetic fibers for 1–1.5 hours

![Graph](image-url)
either in the presence or the absence of glucose was within the range of 0.19 to 0.38 μg/g reported for unstimulated vessels (31). In view of the pharmacological demonstration of different pools of norepinephrine in sympathetic nerves (32), our results do not exclude the possibility that glucose deprivation affects the synthesis or binding of norepinephrine within a pool which would not be revealed by measuring changes in total norepinephrine content. Furthermore, increased turnover of norepinephrine in the nerve fiber may occur independently of changes in tissue norepinephrine content. However, it appears that the enhanced release of the adrenergic transmitter in rat mesenteric arteries produced by glucose deprivation is probably due to either a direct effect of glucose on the release process or depletion of a glucose metabolite(s), which may provide energy for control of the release of the adrenergic transmitter, bearing in mind that additional effects on synthesis or binding of norepinephrine may contribute to this effect of glucose removal.

If the enhanced release of the adrenergic transmitter produced by glucose deprivation is due to depletion of glucose metabolites, then one or more glucose metabolites should inhibit adrenergic transmission. Furthermore, antagonists of glucose metabolism should abolish the inhibitory effect of glucose on adrenergic transmission. The glucose metabolites, pyruvate and lactate, inhibited the potentiated vasoconstrictor response of rat mesenteric arteries to nerve stimulation in the absence of glucose. Since these agents did not alter the basal perfusion pressure and reduced the vasoconstrictor response to nerve stimulation more than they reduced the response to injected norepinephrine, their inhibitory effect on the response to nerve stimulation is most probably due to diminished release of norepinephrine from sympathetic nerve fibers. The failure of these agents to inhibit adrenergic transmission in the presence of glucose provides additional support for their mediation of the inhibitory effect of glucose on adrenergic transmission. Pyruvate appears to be the more likely mediator of this inhibitory effect, since, unlike lactate, which did not affect the potentiated vasoconstrictor response to injected norepinephrine, pyruvate inhibited the response to injected norepinephrine as well as that to nerve stimulation. Furthermore, pyruvate inhibited each adrenergic stimulus to a degree similar to the inhibition produced by glucose.

Other products of glucose metabolism such as amino acids, sugar alcohols, and adenine nucleotides were studied and found wanting as potential mediators of the inhibitory action of glucose on adrenergic neurotransmission. These metabolites were either without effect on adrenergic transmission (sugar alcohols) or their effect could only be demonstrated at very high concentrations, or, if demonstrated at smaller concentrations, the response to nerve stimulation was affected similarly in the absence and the presence of glucose. Adenine nucleotides inhibited adrenergic transmission in mesenteric arteries perfused with glucose-free Tyrode's solution, and ATP reduced the vasoconstrictor response to nerve stimulation to a much greater degree than it reduced the response to injected norepinephrine compared with the other nucleotides (Fig. 4), suggesting that, of these compounds, ATP is the most likely mediator of the inhibitory effect of glucose on adrenergic transmission. Since ATP is rapidly broken down into di- and monophosphate nucleotides (33-35), the latter may contribute to the action of ATP on adrenergic transmission. However, ATP, unlike pyruvate or lactate, produced similar effects on adrenergic transmission in rat mesenteric arteries in the absence and the presence of glucose. Since ATP, ADP, and AMP applied exogenously do not easily penetrate the cell membrane (18), their inhibitory action on adrenergic transmission may not reflect the action of endogenous nucleotides on release of the adrenergic transmitter. ATP is present in relatively large amounts in adrenergic tissues (36-38) and has been shown to be involved in both uptake and release of norepinephrine in isolated adrenergic nerve granules (35). ATP is released together with catecholamines on stimulation of adrenal medullary vesicles and the intact adrenal gland (39). Adrenergic nerves supplying the guinea pig gut and the rabbit aorta and pulmonary arteries after exposure to H-adenosine have been shown to release tritium-labeled compounds. The release of labeled nucleotides as well as norepinephrine is blocked by guanethidine (18, 40). These observations, taken together with our present work, raise the possibility that ATP and other nucleotides released on stimulation of sympathetic nerve fibers modulate sympathetic transmission by inhibiting further release of the adrenergic transmitter. The mechanism by which nucleotides inhibit neurotransmission may involve their ability to decrease the entry of Ca2+ into the nerve terminal; this divalent cation is essential for release of the adrenergic transmitter (41, 42). Since ATP is an efficient chelator of Ca2+ (43), it may alter Ca2+ concentration by chelation or precipitation (44,
This interpretation is supported by our finding that the inhibitory effect of ATP on adrenergic transmission in rat mesenteric arteries is abolished by raising the Ca\(^{2+}\) concentration in the perfusion medium (unpublished results).

An analogue of glucose, 2-DG, which inhibits glycolysis and glucose utilization (20), abolished the ability of glucose to inhibit the potentiated vasoconstrictor response to nerve stimulation produced by glucose deprivation, whereas the inhibition produced by pyruvic acid was unaffected. IAA, which inhibits glycolysis and oxidative metabolism of pyruvate (21), prevented the inhibition of the potentiated vasoconstrictor response to nerve stimulation produced by infusion of either glucose or pyruvate. Prevention by IAA of the inhibitory action of glucose or pyruvate on potentiated adrenergic transmission could, in turn, be restored by increasing the concentration of either glucose or pyruvate. The possibility that IAA may act by inhibiting the entry of glucose into the cell seems unlikely, since IAA does not affect cell permeability to monosaccharides (46). These observations with 2-DG and IAA constitute the most persuasive evidence that one or more metabolites of glucose, particularly pyruvate, may be involved in the regulation of the release of the adrenergic transmitter.

The inhibitory effects of glucose and its metabolite(s) on release of the adrenergic transmitter in mesenteric arteries were also investigated in terms of possible interactions with other regulatory mechanisms. Hedqvist (23) has reported that prostaglandins, which are released on stimulation of adrenergic nerves, in turn, reduce the release of the adrenergic transmitter. In the present study, prostaglandins did not inhibit but rather potentiated the vasoconstrictor responses to nerve stimulation and to injected norepinephrine both in the presence and in the absence of glucose. Moreover, infusion of either indomethacin or meclofenamate, which inhibits synthesis of prostaglandins (24), did not reveal an inhibitory action of endogenous prostaglandins on adrenergically induced vasoconstriction of mesenteric blood vessels. If endogenous prostaglandins in this vascular bed modulated the vasoconstrictor response to nerve stimulation and exogenous norepinephrine, then augmentation of this response should have occurred after inhibition of prostaglandin synthesis by antiinflammatory drugs. This augmentation was not observed; rather, inhibition of the vasoconstrictor response occurred during infusion of meclofenamate and indomethacin due to direct effects of the drugs.

The inhibitory effect of glucose and its metabolite(s) on release of the adrenergic transmitter is not due to stimulation of inhibitory muscarinic receptors either presynaptically, excitation of which would diminish the release of norepinephrine from sympathetic fibers, or postsynaptically, stimulation of which would reduce the vasoconstrictor effect of norepinephrine released from nerve fibers (9, 25, 26). The vasoconstrictor response to nerve stimulation was not altered by concentrations of atropine which abolished the inhibitory effect of acetylcholine (9), and the inhibitory effect of glucose and pyruvate was not affected. Excitation of \(\beta\) receptors postsynaptically was also excluded as a determinant of the inhibitory action of glucose on facilitated adrenergic transmission, since \(\beta\)-receptor blockade by propranolol did not prevent this effect of glucose or its metabolites. Stimulation of presynaptic \(\alpha\) receptors, which reduces release of norepinephrine from sympathetic fibers (47), cannot be excluded as a possible mechanism for the inhibitory action of glucose, since phenoxybenzamine reduces or abolishes the vasoconstrictor responses to nerve stimulation and to injected norepinephrine (7). Of the various possible inhibitory mechanisms investigated, only glucose metabolites seemed to be involved in the inhibitory effect of glucose on the release of norepinephrine from the sympathetic fibers in rat mesenteric arteries. Glucose metabolites may act by serving as an energy source for the mechanism which regulates release of the adrenergic transmitter or by exerting a direct inhibitory effect on the release process, although an additional effect on binding or synthesis of norepinephrine cannot be excluded. Since the inhibitory action of pyruvate on adrenergic transmission was only demonstrated after its facilitation by glucose withdrawal and was prevented by IAA, which affects its utilization, pyruvate and perhaps the products of its metabolism are probable mediators of the modulatory action of glucose on adrenergic transmission in rat mesenteric arteries.

Since release of the adrenergic transmitter is also affected by changes in the ionic content of the extracellular fluid (28), the inhibitory action of glucose on the release of norepinephrine was studied in terms of a possible relationship to ions which are known to modify neurotransmission. To invest an ion with a major role in the inhibitory action of glucose on adrenergic transmission rests on the demonstration that changing the concentration of the ion in the absence of glucose affects the potentiated vasoconstrictor response to nerve stim-
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In the absence of glucose, the entry of Na⁺ into the nerve fiber presumably increases on depolarization of the adrenergic nerve terminal by nerve impulses. One of the consequences of increased entry of Na⁺ into the nerve which might explain enhanced release of norepinephrine is an effect on Ca²⁺. Increased intracellular Na⁺ may facilitate mobilization of intracellular Ca²⁺ or stimulate Ca²⁺ influx, as has been shown in the squid axon (51) as well as for release of the cholinergic transmitter and catecholamines from the adrenal gland (53).

If, in the absence of glucose, release of the adrenergic transmitter by nerve stimulation is increased by raising Na⁺ concentration, then an opposite effect would be expected when Na⁺ concentration is decreased. However, lowering the Na⁺ concentration produced variable effects on the response to nerve stimulation and markedly augmented the response to injected norepinephrine. The latter would have obscured the predicted effect of decreased Na⁺ on the vasoconstrictor response to nerve stimulation. Potentiation of the responses to injected norepinephrine produced by lowering Na⁺ concentration is presumably due to interference with reuptake of norepinephrine by the nerve ending. Uptake of norepinephrine across the neuronal membrane and its retention in the nerve terminal have been shown to be dependent on the presence of Na⁺ in the medium (54-56).

If enhanced release of the neurotransmitter produced by glucose deprivation is due to increased entry of Na⁺ into the nerve, which affects the availability of Ca²⁺ to the release process, then altered K⁺ levels may also affect the release process in view of the coupled transport of Na⁺ and K⁺ across cell membranes (57). Increased K⁺ concentration inhibited the potentiated vasoconstrictor response of mesenteric blood vessels to nerve stimulation only in the absence of glucose. Since the basal perfusion pressure remained unaltered and the equiconstrictor response to injected norepinephrine was reduced, this potentiation must be due to increased release of norepinephrine from sympathetic fibers. A hyperosmotic effect of elevated Na⁺ was excluded as a determinant of the potentiation, since the addition of either mannitol or sucrose, in concentrations equimolar to elevated Na⁺, reduced the vasoconstrictor responses to both adrenergic stimuli. Potentiation of the response to nerve stimulation produced by increased Na⁺ in the absence of glucose but not in its presence suggests that glucose may exert its inhibitory effect on release of the adrenergic transmitter by maintaining intraneuronal levels of Na⁺.
were unaltered, respectively. Increased K+ has been shown to release catecholamines in the absence of nerve stimulation (58, 59). Since changes in K+ concentration affected the vasoconstrictor response to nerve stimulation differently in the presence and the absence of glucose, modulation by glucose of the release of the adrenergic transmitter may be related not only to maintaining Na+ levels but also K+ levels intraneuronally, i.e., by reducing Na+ and increasing K+. In support of this interpretation, decreased K+ concentration enhanced the release of norepinephrine. Lowering the K+ concentration of the perfusion fluid has been reported to increase the acetylcholine-induced release of norepinephrine from the isolated rabbit heart when the output of norepinephrine is kept at a submaximal level by decreasing the external Ca2+ concentration (60). In addition, removal of K+ from the medium enhances efflux and reduces retention of 3H-norepinephrine in prelabeled rat ventricle slices (55).

The mechanism by which one or more metabolites of glucose maintain intraneuronal concentrations of Na+ and K+ may involve their direct action on the adrenergic nerve terminal as well as their ability to serve as an energy source for Na+, K+-activated adenosinetriphosphatase (ATPase) (61, 62). Inhibition of Na+, K+-activated ATPase by ouabain results in increased Na+ and reduced K+ intracellularly and is associated with increased output of catecholamines from the bovine adrenal gland (63). In rat mesenteric arteries, ouabain also augments the vasoconstrictor response to sympathetic nerve stimulation (unpublished results). Thus, glucose may affect the release of adrenergic transmitter by maintaining intraneuronal levels of Na+ and K+ which in turn affect the availability of Ca2+ to the release process.

Like glucose, other sugars may also affect release of the adrenergic transmitter. After removal of glucose, other hexoses such as fructose, mannose, and galactose, and a pentose, xylose, reduced the potentialized vasoconstrictor response to nerve stimulation to a greater degree than they reduced the response to injected norepinephrine. Variations in the degree of the inhibitory effect of these sugars on release of the adrenergic transmitter is probably due to differences in their ability to penetrate the neuron as well as to differences in their metabolism (64, 65). Glucose also inhibits release of the adrenergic transmitter from sympathetic fibers of tissues other than rat mesenteric arteries. Thus, we have recently observed in the isolated perfused rat kidney, the guinea pig vas deferens, and the rabbit ear artery and vein that the responses of these tissues to postganglionic sympathetic nerve stimulation are markedly potentiated by glucose deprivation and restored to normal by the addition of glucose to the perfusion fluid (unpublished work). These observations and the results of the present study suggest to us that glucose and some other sugars play an important role in modulating the release of the adrenergic transmitter from postganglionic nerve fibers presumably by maintaining the concentration of one or more metabolites, such as pyruvate, which mediate this action. A decrease in pyruvate and possibly other products of glucose metabolism consequent to removal of glucose results in changes in the concentrations of Na+ and K+ within the neuron which in turn enhance the availability of Ca2+, thereby, increasing the release of the adrenergic transmitter.

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KAFAIT U. MALIK and JOHN C. McGIFF

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