Neurogenic Vasodilation of Cat Cerebral Arteries

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SUMMARY Transmural nerve stimulation (TNS) with 0.3-msec pulses between 1 and 25 Hz dilated cat cerebral artery segments in the presence of active muscle tone. Maximum vasodilatation occurred at 8 Hz. The dilator response to exogenous acetylcholine, but not to TNS, was abolished by atropine. Neither physostigmine nor hemicholinium affected the dilator response to TNS, which persisted after administration of guanethidine, phenoxybenzamine, propranolol, reserpine, and chronic sympathectomy. However, it was abolished by tetrodotoxin and cold storage. When examined histochemically, cat and rabbit cerebral arteries exhibited a rich plexiform distribution of acetylcholinesterase which was not affected appreciably by sympathetic denervation. These results suggest that vasodilation is not mediated through modification of sympathetic activity. They also indicate the existence of a nonadrenergic, possibly noncholinergic, vasodilator innervation in cat cerebral arteries. Preliminary studies suggest that the transmitter is not histamine, ATP, prostaglandins, γ-aminobutyric acid, dopamine, or serotonin. The cat cerebral artery segments contrast with the isolated rabbit cerebral arteries which predominantly constrict in response to TNS and show a small dilator response.

THERE IS AMPLE evidence that cerebral blood vessels of several species receive both sympathetic and nonsympathetic innervation. Based on anatomical and physiological observations, it seems likely that the nonsympathetic neurons are part of the parasympathetic nervous system, that they are dilator nerves, and that they run via the greater superficial petrosal nerve to the cerebral blood vessels. Mchedlishvili and Nikolashvili have stated that this dilator innervation is important in regulating cerebral blood flow, although no significant changes in this parameter resulted when the petrosal nerve or vagus nerve was sectioned. When examined histochemically, cat and rabbit cerebral arteries exhibited a rich plexiform distribution of acetylcholinesterase which was not affected appreciably by sympathetic denervation. These results suggest that vasodilation is not mediated through modification of sympathetic activity. They also indicate the existence of a nonadrenergic, possibly noncholinergic, vasodilator innervation in cat cerebral arteries. Preliminary studies suggest that the transmitter is not histamine, ATP, prostaglandins, γ-aminobutyric acid, dopamine, or serotonin. The cat cerebral artery segments contrast with the isolated rabbit cerebral arteries which predominantly constrict in response to TNS and show a small dilator response.

Methods

Tissues were removed from exsanguinated cats and rabbits of either sex for in vitro examination. Adult cats (2.2-3.5 kg) were anesthetized with pentobarbital (50 mg/kg, i.p.), and adult white rabbits (2-3 kg) were stunned prior to exsanguination. The entire brain, with pial arteries attached, and also the distal saphenous artery were removed rapidly and placed in Krebs bicarbonate solution equilibrated with 95% O2 and 5% CO2 at room temperature. The composition of the Krebs solution was (mm): Na+, 144.2; K+, 4.9; Ca2+, 1.6; Mg2+, 1.2; Cl-, 126.7; HCO3-, 25.0; SO42-, 1.19; glucose, 11.1; and calcium disodium ethylenediamine tetraacetate, 0.023. The vessels were dissected and cleaned of surrounding tissue, using a dissecting microscope.

Ring segments (4 mm long) of the arteries were mounted in isolated tissue baths containing 30 ml of Krebs bicarbonate solution at room temperature according to the method of Bevan and Osher. Ten minutes after artery segments were set up, the temperature of the Krebs bicarbonate solution was increased gradually over a 15-minute period and maintained at 37°C. Resting tension was adjusted to 0.5 g, and a period of 1 hour was allowed for equilibration. A pair of stimulating electrodes was arranged, one on either side of the vessel, for transmural nerve stimulation (TNS). Trains of 100 or 200 biphasic rectangular pulses of 0.3-msec duration were delivered at supramaximal voltage, using a Grass stimulator model S5. Unless otherwise stated, drugs were added directly to the tissue bath 10 minutes prior to testing.

Frequency Response Curves

The dilator responses of a given intracranial artery preparation to a set of TNS at 1, 2, 4, 8, and 25 Hz in random sequence were elicited without changing the bath solution. A period of 6 minutes was allowed between each stimulation. At the end of each experiment, individual dilator responses to TNS were expressed as a percent of the relaxation caused by papaverine (100 μM).
Catecholamine and Acetylcholinesterase Histofluorescence

Freshly dissected whole-mount preparations of arteries adjacent to those used for the tissue bath study were treated with glyoxylic acid to induce catecholamine fluorescence. Acetylcholinesterase was demonstrated in whole-mount preparations of arteries using a modification of the “direct-coloring” thiocholine technique of Karnovsky and Roots. Artery segments were air-dried on glass slides, then fixed for 30 minutes in formalin-sucrose-ammonia. All other steps in the staining procedure prior to dehydration were carried out in 0.1M aqueous sodium hydrogen maleate (pH 6.0). The specimens were rinsed in maleate buffer (pH 6.0) briefly, then preincubated at room temperature for 30 minutes in either one or both of the enzyme inhibitors IsoOMPA (tetraisopropyl pyrophosphoramide), 10^{-5} M, and BW284C51 [1,5-bis(N-allyl-N-dimethyl-4-ammonium phenyl)pentan-5-one dibromide], 3 × 10^{-5} M. The specimens were again rinsed briefly with maleate buffer, then incubated for 1 hour at room temperature in a medium containing acetylthiocholine iodide (2 mM), sodium citrate (5 mM), copper sulfate (6 mM) and potassium ferricyanide (0.5 mM). For those specimens which had been preincubated in BW284C51, the compound was included in the incubation medium at the same concentration. At the end of incubation, the specimens were rinsed and dehydrated through graded concentrations of ethanol and mounted for light microscopic examination.

Sympathetic Denervation

Three cats were treated with reserpine [Serpasil (3 mg/kg/day, intraperitoneally)] for 2 days and were killed 24 hours after the last dose. Superior cervical ganglionectomy was performed bilaterally on two cats and unilaterally on two other cats. Some arterial segments from control, reserpine-treated, and sympathetically denervated animals were stored in Krebs bicarbonate solution at 4°C for 7 days to achieve cold storage denervation. The disappearance of catecholamine fluorescence was taken as an indication of complete adrenergic denervation.

Statistical Methods and Drugs

The data were evaluated statistically by Student’s t-test for paired samples. The 0.05 level of probability was accepted as significant. The following drugs were employed: l-norepinephrine bitartrate (Calbiochem), phenoxybenzamine hydrochloride (Smith, Kline and French), phenolamine methanesulfonate (Ciba), propranolol hydrochloride (Ayerst), dopamine hydrochloride (Calbiochem), tetrodotoxin (TTX) (Sankyo-Tokyo), reserpine (Ciba), guanethidine sulfate (Ciba), bretylium tosylate (Burroughs Wellcome), acetylcholine chloride (Calbiochem), atropine sulfate (Merck), physostigmine salicylate (Merck), hemicholinium bromide (Fisher), serotonin creatinine sulfate (Calbiochem), histamine dihydrochloride (Pfamstiehl Chemical Co.), pyridine maleate (Merck), betiamide (SK&F), isoproterenol hydrochloride (Sigma), y-aminobutyric acid (Calbiochem), adenosine triphosphate (Sigma), acetylthiocholine iodide (Sigma).

Results

Response of Cerebral Arteries to Transmural Nerve Stimulation

The basilar, anterior, middle, and posterior cerebral, posterior communicating and anterior cerebral arteries without exception relaxed upon TNS (Fig. 1). This was observed only if the vessel maintained an active muscle tone, either spontaneous and intrinsic (16 out of 50) or induced by dopamine (10-30 μM), norepinephrine (NE) (30 μM) or serotonin (5HT) (0.7-7 μM). The dilator responses to TNS, but not to exogenously applied acetylcholine (Ach) (0.3 μM) and papaverine (43 μM), were abolished by TTX (0.6 μM) (n = 40) or cold storage (n = 5) (Fig. 2). The dilator response could be elicited by pulses as short as 0.1 msec in duration, suggesting that the dilator responses to TNS were due exclusively to excitation of intramural nerves.

Characteristics of Neurogenic Vasodilator Responses

The magnitude of dilator responses to TNS was a function of stimulation frequency (Fig. 1). The maximum relaxation was achieved at 8 Hz (Fig. 3). Irrespective of the cause of active tone, at stimulation frequencies about 4 Hz, a fast relaxation was followed by a biphasic recovery pattern. An initial fast phase was followed by a slower phase. At lower frequencies, only the slower phase of recovery was observed.

Effect of Sympathectomy and Reserpinization on Neurogenic Vasodilator Responses

One week after removal of one superior cervical ganglion, ipsilateral cerebral arteries, and artery segments taken from reserpine-pretreated cats exhibited no catecholamine fluorescence following excitation of intramural nerves.
choamine fluorescence. Both artery preparations dilated in response to TNS (Fig. 1b and Fig. 4). The size of the dilator response, expressed as a percentage of the maximum response to papaverine (100 \( \mu M \)), was not significantly different from that of control preparations (Fig. 3).

**Effect of Drugs that Interfere with Adrenergic Transmission on the Neurogenic Vasodilator Responses**

The dilator responses to TNS were not affected by adrenergic neuronal blocking agents, guanethidine (5 \( \mu M \)) and bretylium (7.2 \( \mu M \)), nor by \( \alpha- \) and \( \beta- \)adrenergic receptor blocking agents, phenoxbenzamine (PBZ) (3.3–10 \( \mu M \)), phentolamine (10 \( \mu M \)), and propranolol (2 \( \mu M \)) (Fig. 5a and 5b). Isoproterenol (0.3 \( \mu M \)) caused dilation which was abolished completely by propranolol (3 \( \mu M \)).

**Effect of Drugs that Interfere with Cholinergic Transmission on the Neurogenic Vasodilator Responses**

It has been reported previously\(^1\) that acetylcholine (0.01–10 \( \mu M \)) causes dilation of the cat cerebral artery. This is abolished by atropine (0.43 \( \mu M \)), whereas the response to TNS is unaltered. This result was supported further by experiments showing that the dilator response to TNS was uninfluenced by atropine (14 \( \mu M \)) which abolished dilation to 100 times the dose of Ach equipotent to TNS at 8 Hz.

Physostigmine (7.2–8 \( \mu M \)), which slightly increased the resting vessel tone, did not potentiate the dilator response to TNS at 2, 4, and 8 Hz (Fig. 6a; \( n = 3 \)) \((P > 0.5)\) when this was expressed as a percentage of the maximum relaxation to papaverine. Hemicholinium (100–300 \( \mu M \)), which did not affect the contractile response to NE, serotonin, or dopamine, did not abolish the dilator response to TNS at 8 Hz after 4 hours of exposure (Fig. 6b; \( n = 3 \)) \((P > 0.4)\). When active muscle tone was induced and maintained by a contraction-producing high concentration of Ach (55 \( \mu M \)), TNS at 8 and 25 Hz still caused relaxation in control and in sympathetically denervated arteries.

**The Effects of Histamine, Adenosine Triphosphate, Prostaglandin and \( \gamma \)-Aminobutyric Acid (GABA)**

Histamine caused dilation of actively contracted cat cerebral artery segments. Such dilator responses to histamine (16 \( \mu M \)) were abolished by betiamide (15 \( \mu M \)), indicating that an \( H_1 \) receptor was involved. The dilator responses to TNS at 8 Hz were unaffected by betiamide (15 \( \mu M \)) or pyrilamide (7.4 \( \mu M \)), an \( H_2 \) receptor blocking agent, or both together (Fig. 7A).

Adenosine triphosphate (0.005–1 \( \mu M \), \( n = 3 \)) caused a slow dilation of the cat cerebral arteries. The time course of the slow dilation contrasted with the brisk neurogenic response seen at 4, 8, and 25 Hz (Fig. 7B). At higher concentrations, ATP (6–10 \( \mu M \)) caused constriction of the artery. In the two cases tested, when active muscle tone was maintained by ATP (10 \( \mu M \)), TNS still caused relax-
Dilator responses of cat basilar artery to TNS at different frequencies before and after administration of guanethidine (6 μM). Guanethidine did not affect either the shape or the magnitude of dilator responses.

FIGURE 5

Dilator responses of cat posterior communicating artery to TNS at different frequencies before and after administration of phenoxybenzamine (PBZ; 3.3 μM). PBZ abolished the response to NE but not the dilator responses to TNS in the presence of intrinsic muscle tone.

Response of Cat Saphenous and Rabbit Basilar Arteries to Transmural Nerve Stimulation

In view of the predominantly vasoconstrictor response to TNS previously reported for the rabbit basilar artery, this vessel was reexamined for a dilator response. In the absence of active muscle tone, the basilar artery constricted in response to TNS. In the presence of active muscle tone, 77 out of 150 artery preparations dilated slightly after constriction in response to TNS. The constrictor, but not the dilator, component was abolished by guanethidine (5 μM) or bretylium (7.2 μM).

FIGURE 6

Dilator responses of cat middle cerebral artery to TNS before and after physostigmine (8 μM). The dilator responses to TNS at 2 Hz were not potentiated. The middle cerebral artery had been sympathetically denervated by removal of the superior cervical ganglia 6 days prior to the experiment. Dilator responses were not affected by hemicholinium in the course of a 4-hour exposure. Active muscle tone was produced by serotonin (7.4 μM). The middle cerebral artery had been sympathetically denervated by removal of the superior cervical ganglia 6 days prior to the experiment.
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PGE, pyrilamine (7.4 \mu M), 0.5 Hz, Dopamine (30 \mu M) 30 Hz

FIGURE 7

A: Effect of betamidine and pyrilamine on the dilator responses of cat middle cerebral artery segment to histamine and TNS. Betamidine (15 \mu M) and pyrilamine (7.4 \mu M) abolished dilator responses to histamine (55 \mu M). Dilator responses to TNS at 25 Hz were unaffected (5HT = serotonin; NE = norepinephrine). B: Dilator response of cat middle cerebral artery to ATP. In the presence of active muscle tone, ATP causes relaxation of the artery. The time course of the dilator response is much slower than that induced by TNS at 4, 8, and 25 Hz. At a higher dosage, ATP (55 \mu M) caused constriction of the artery, even in the presence of active muscle tone.

The dilator response was unaffected by atropine (5 \mu M, n = 3).

To show that the presence of a dilator component is not a species-specific phenomenon unique to the sympathetic innervation of cat blood vessels, the neurogenic response of the cat saphenous artery was investigated. Saphenous artery segments invariably constricted in response to TNS, even in the presence of active muscle tone. This response was abolished by guanethidine (2-5 \mu M) (Fig. 9).

Acetylcholinesterase in Cat and Rabbit Cerebral Arteries

A plexiform pattern of histochemically demonstrated acetylcholinesterase was seen in both cat and rabbit cerebral arteries. It was not changed by chronic sympathetic denervation (Fig. 10).

Discussion

Since the early findings of Forbes and Wolff, Chorobski and Penfield, and Cobb and Finesinger for the cat, there have been many reports providing evidence of cerebral vessel dilator innervation. However, several investigators have been unable to demonstrate neurogenic dilation in vivo when the petrosal nerve or vagus nerve was electrically stimulated.

During a study of the neurogenic responses of the isolated rabbit cerebral artery, we found that some preparations exhibited a small dilation following a marked vasoconstriction. Both responses were abolished by TTX. In contrast to the rabbit, all cat cerebral arteries examined showed only dilation in response to TNS. These responses were abolished by TTX and by previous cold storage of the artery, indicating that they were of neurogenic origin.

Recently, morphological observations have suggested that there are two types of nerve endings in the walls of cerebral arteries from several species. One contains dense granular vesicles and is assumed adrenergic; the other, observed even in animals pretreated with 6-hydroxydopamine or 5-hydroxydopamine, contains agranular vesicles and is generally assumed to be cholinergic. This latter conclusion is supported by the presence of acetylcholinesterase in cerebral blood vessels, presumably in association with a ground plexus and the findings that acetylcholine administered intravascularly dilates pial arteries or increases cerebral blood flow, that carbachol applied locally dilates pial arteries, and that autoregula-

Figure 8: Effect of indomethacin on the dilator responses to TNS. The dilator responses to TNS in the presence of active muscle tone induced by serotonin (5HT) were not affected by indomethacin (8-25 \mu M). Prostaglandin E1 caused a dilation.

Figure 9: Responses of cat saphenous artery branch to TNS. In the absence of muscle tone, the artery constricts to TNS. The contractile responses were abolished by guanethidine (2 \mu M). In the presence of active muscle tone, there was no dilator response to TNS even after guanethidine.
Figure 10 Whole-mount acetylcholinesterase preparation of cat cerebral arteries. A: Control middle cerebral artery. B: Ipsilateral vessel after chronic superior cervical ganglionectomy. Calibration, 50 μm.

Circulatory cerebral vasodilation following a decrease in systemic arterial blood pressure is blocked by atropine. These results taken together suggest that acetylcholine is released to cause vasodilation when the nonadrenergic nerve is excited. However, no direct evidence has been presented.

The distribution and intensity of acetylcholinesterase found in this study on cat and rabbit cerebral arteries were not altered by obtaining chronic sympathetic denervation. The presence of acetylcholinesterase is not necessarily indicative of cholinergic neurons; in fact, acetylcholinesterase has been seen in association with apparently typical sympathetic neurons. However, the observation that the amount of enzyme observed was unaltered by superior cervical ganglionectomy does suggest that the enzyme is associated with the nonsympathetic innervation.

The optimal stimulation frequency for dilator response of cat cerebral arteries is about 8 Hz. This is a characteristic of other parasympathetic, cholinergic nerves. The failure of guanethidine, bretylium, propranolol, phenoxybenzamine, phentolamine, reserpine, and sympathectomy to affect the dilator response to TNS indicates that neurogenic vasodilation of cat cerebral arteries is independent of the adrenergic sympathetic outflow. Finally, Ach caused a dilation of the cerebral arteries.

Although the above results are compatible with cholinergic dilator mechanisms, the dilator responses to TNS were resistant to atropine, a cholinergic blocking agent, in concentrations much higher than necessary to abolish the dilator effect of Ach.

The failure of atropine to abolish neurogenic vasodilation does not eliminate Ach as a transmitter. The
smooth muscle of the urinary bladder receives cholinergic parasympathetic innervation that is not blocked by atropine. However, the contraction of urinary bladder smooth muscle to nerve stimulation is abolished by hemicholinium and potentiated by physostigmine. Neither physostigmine nor hemicholinium influenced the neurogenic vasodilator response of the cat cerebral arteries when used in these same concentrations. Both rabbit and cat cerebral arteries showed strong histochemical staining for acetylcholinesterase. There was marked disparity between the magnitude of the dilation in the two species. Both responded to exogenous Ach. It is well recognized that the presence of small clear agranular vesicles is by no means indicative of a cholinergic neuron. There is evidence that such vesicles occur in electrotonic synapses and in sensory terminals. Recent evidence suggests that cerebral vessel dilator fibers originate in the midbrain and that the cerebral blood flow increase in the dog during medullary electrical stimulation is insensitive to atropine.

There is much evidence that ATP is the noncholinergic, nonadrenergic inhibitory transmitter in the gut. It has been suggested that neurogenic ATP initiates the complex neurogenic contraction of the smooth muscle of urinary bladder. The possibility that ATP is the vasodilator transmitter in cat cerebral artery was considered. The ATP-induced cerebral artery vasodilation was inconsistent and was slower when compared with the dilator response to TNS; the latter response still was seen in the presence of a contraction-producing dose of exogenous ATP. Thus, the possibility of ATP as the dilator transmitter in cat cerebral artery is unlikely. Dipyridamole (0.1-1 μM) could not be used in this study because it always caused relaxation and abolished the active muscle tone induced by norepinephrine or serotonin.

Histamine has been reported to be the neurotransmitter for hindlimb vasodilation. It dilated the cat cerebral artery segments in the presence of active muscle tone (Fig. 7). However, betamamide, which abolished the histamine-induced dilation, did not affect the dilator response to TNS.

Prostaglandin has been reported to be involved in the vasodilatation resulting from stimulation of the sympathetic nerve to dog hindlimb and in rabbit adipose tissue. PGE, dilated the cat cerebral artery segment in the presence of active muscle tone, but the TNS-induced dilation was not influenced by indomethacin (25 μM).

Dopamine and 5HT have been reported to mediate neurogenic vasodilation in the kidney and intestine respectively. Both dopamine and 5HT caused only contraction of the cerebral artery in either the presence or absence of active muscle tone. As mentioned previously, dilator responses were elicited in the presence of active muscle tone induced by 5HT and dopamine. Furthermore, vasodilation was not abolished by PBZ.

Toda observed a nicotine-induced dilation of canine cerebral arteries. This effect appeared to be dependent on the innervation of the blood vessel. He showed that it was not mediated through α-adrenergic and cholinergic mechanisms. Neither his study nor our own provides positive evidence concerning the nature of the transmitter. It is possible that the transmitter is a hitherto unrecognized chemical mediator. Alternatively, the postsynaptic receptors may be inaccessible to either exogenous agonist or antagonist. Since an atypical α-adrenergic receptor has been described in both cat and rabbit cerebral arteries, the dilator transmission process may involve a well-recognized transmitter but not exhibit typical features due to some local and unique property.

On the basis of our studies in vitro, the dominant response of the cat cerebral arteries is vasodilation. This concept is contrary to that of Edvinsson and Owman. These investigators observed only vasoconstriction of cat cerebral artery rings in response to transmural electrical stimulation. In these studies, a longer pulse duration (1 msec), compared with 0.3 msec used in our experiments was employed for field stimulation. It is true that, in our study, when pulse duration was increased to 1 or 3 msec, vasoconstriction was seen (see Fig. 2). However, this constriction response was not abolished by TTX (1.8 μM) and this suggests that the constriction probably was due to direct excitation of the smooth muscle cells. Edvinsson and Owman did not demonstrate that the stimulation excited only neural elements.

Edvinsson and Owman have suggested that cerebral vasodilation is brought about by inhibition of ongoing sympathetic vasoconstriction, i.e., is mediated through the sympathetic nervous system. It is interesting, however, that cat cerebral artery segments only dilated in response to TNS (0.3 msec), whereas TNS with the same parameters always constricted the saphenous artery (Fig. 9). It is possible that the excitation of constrictor nerve was masked by concurrent dilator nerve activation in the cerebral artery in the present experiments. However, this possibility is unlikely in view of the failure of chronic sympathectomy, reserpine, guanethidine, and PBZ to affect the extent and time course of TNS-induced vasodilation and the frequency-dilator response relationship. Using microsphere technique and 85 Kr injection technique to measure cerebral blood flow, Alm and Bill and Waltz et al concluded that sympathetic innervation contributes little to the normal regulation of cat cerebral blood flow.

Our results suggest that, if vasodilator fibers are functionally significant, their role is species-dependent. If one assumes that both sympathetic vasoconstrictor and non-sympathetic vasodilator fibers supply the cerebral vessels of both rabbit and cat, the former appear to be potentially dominant in the rabbit and the latter in the cat.

The vasodilator transmitter and the receptor upon which it acts have not been identified. If acetylcholine is the transmitter, the transmission does not exhibit the usual features. Nevertheless, the possible physiological function of a dilator innervation should be considered in this species, especially because there is little evidence from these studies that the sympathetic innervation is functionally important.

References

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