Electrical and Mechanical Characteristics of Vascular Smooth Muscle Response to Norepinephrine and Isoproterenol

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ABSTRACT

Effects of norepinephrine (NE) and isoproterenol on simultaneously recorded electrical and contractile activity in rat portal vein were studied using a sucrose-gap technique. This vascular smooth muscle shows spontaneous phasic contractions correlated with bursts of action potentials. Norepinephrine (10^{-9} to 10^{-7} w/v) increases the duration of the bursts and shortens the interval between bursts after an initial period of continuous spike discharge. The tension response is greater than can be accounted for by the increase in electrical activity. High NE concentrations (10^{-5}) produce depolarization, decrease of spike amplitude, or even abolition of spikes and maintained contractions. Norepinephrine increases contracture tension of K^+-depolarized portal vein without changing membrane potential. Electrical and mechanical activity is reinitiated in preparations inactivated by elimination of Ca^{2+}; this may be due to release of bound calcium. Phenoxybenzamine abolishes the above NE responses. The typical response to isoproterenol (10^{-9} to 10^{-7}) in a normal ionic environment consists of moderate depolarization, decreased burst duration, but increased frequency of bursts and inhibition of tension development which is not simply correlated with the change in electrical activity. This pattern resembles that produced by lowering [Ca^{2+}]_o. Contracture tension in high [K^+]_o is reduced by isoproterenol without changes in membrane potential. These responses to isoproterenol are abolished by propranolol. At high concentrations of isoproterenol (10^{-5}) the inhibitory responses are not sustained, but revert to a pattern of activity resembling that induced by NE.

ADDITIONAL KEY WORDS membrane potential phenoxybenzamine propranolol depolarized smooth muscle role of calcium rat portal vein

Catecholamines produce either vasoconstriction or vasodilatation in intact vascular beds, the direction of the response depending primarily on the type and the dose of the agent used and on the vascular bed studied. The cellular mechanisms responsible for these opposite reactions of vascular smooth muscle to adrenergic agents are insufficiently understood. A better understanding of these excitatory and inhibitory actions would require, among other things, a more complete knowledge of the electrophysiological events associated with the mechanical changes.

In the previous article in this issue (4), we have described the technique used here for simultaneous recording of electrical and mechanical activity of the isolated rat portal vein and the responses of this preparation to changes in ionic composition of extracellular fluid. The present paper describes the electrical and mechanical responses to norepinephr-
rine and isoproterenol and places special emphasis on the inhibitory effects produced by the latter drug.

Results pertaining to the current study have been presented in preliminary form (1, 2).

Methods

Preliminary experiments with recording of only mechanical activity were done to obtain a general orientation about the drug effects. The responses were then subjected to a more detailed analysis in experiments with simultaneous recordings of electrical and mechanical events. Portal veins of rats weighing 150 to 250 g were used. The animals were killed by a blow on the neck; the abdomen was opened and the portal vein dissected free from peritoneum and fat. A section, 5 to 10 mm long, was cut out and mounted in a bath of physiological salt solution.

The "normal" Krebs solution used in all the electrophysiological experiments and in most of the preliminary mechanical experiments had the following composition in millimoles per liter: Na\(^+\), 137.5; K\(^+\), 5.94; Ca\(^{2+}\), 2.49; Mg\(^{2+}\), 1.19; Cl\(^-\), 134.1; HCO\(_3\)\(^-\), 15.5; H\(_2\)PO\(_4\)\(^-\), 1.19; glucose, 11.5. A physiological salt solution of slightly different composition (3) was used in some of the mechanical experiments. This latter medium contained 0.026 mM calcium disodium versenate, which largely prevents degradation of catecholamines. Relatively short periods of drug exposure were used in the present study and the results obtained with norepinephrine and isoproterenol were in all important respects similar in the two solutions. The solutions were bubbled with a gas mixture of 97% O\(_2\) and 3% CO\(_2\) and temperature was kept at 37°C.

The bath used in the mechanical experiments had a volume of 30 ml. The mechanical activity of the longitudinal musculature of the portal vein was recorded isometrically with a force-displacement transducer (Grass FT 03) operating an ink-writing oscillograph (Grass polygraph). The pas-
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dynes

1000  5 mv

500

0

10 sec

FIGURE 2

Response of portal vein to a high norepinephrine concentration. a = Control in normal Krebs solution, b = Immediate effects of norepinephrine 10 μg/liter, c = After 4 min of drug exposure, d and e = After 3 and 10 min recovery in normal Krebs solution, respectively. The zero level of the tension scale and the 5 mv marker are as described in the legend to Figure 1.

Sive tension applied to the muscle in this series of experiments varied between 200 and 400 dynes. The muscle was allowed about 1 hr recovery in the bath at this passive tension before any drugs were added. The method used for simultaneous recordings of mechanical and electrical activity of the portal vein has already been described (4).

In these experiments the effects of norepinephrine and isoproterenol on electrical and mechanical activity of the portal vein were studied not only in normal Krebs solution, but also in solutions with altered K+ and Ca2+ concentrations. The activity of the portal vein in these modified solutions has been described (4).

The following drugs were used: 1-norepinephrine bitartrate (Norexadrine, Astra), 1-isoproterenol sulphate, phenoxybenzamine HCl (Dibenzyline, Smith, Kline and French) and 1-isopropylamino-3-(1-naphthoxy)propanol-(2) HCl (Propranolol).

Fresh solutions of isoproterenol and propranolol were prepared from the pure substances, whereas dilutions of norepinephrine and phenoxybenzamine were made from the commercial stock solutions. Concentrations in weight/volume units are expressed for norepinephrine as base and for the remaining three drugs as the respective salts.

The results reported here were obtained in experiments on a total of 59 portal vein preparations, 25 of which were used for mechanical experiments and 34 for simultaneous recording of electrical and mechanical activity.

Results

I. NOREPINEPHRINE

1. In Normal Krebs Solution. The pattern of electrical and mechanical activity recorded from the portal vein in normal Krebs solution is illustrated in Figure 1a. Action potentials of 2 to 10 mv appeared in this experiment in bursts of 3 to 8 sec duration. Simultaneous with each burst of action potentials there was a contraction of 300 to 500 dynes.

When norepinephrine was added to the Krebs solution in a concentration of 100 μg/liter, the pattern of electrical activity was changed into one of continuous spike discharge, and at times phasic contractions be-
came tonic (Fig. 1b). The force of the mechanical response appeared to increase more than could be accounted for by the change in the pattern of spike discharge.

After 3 to 4 min had elapsed following addition of norepinephrine to the Krebs solution, the pattern of intermittent discharge was resumed, but the duration of the bursts was greatly increased and the intervals between them decreased (Fig. 1c). Here the increased force of the mechanical response was very pronounced, and the possibility of an effect on tension not caused by potential changes cannot be excluded. In the periods of inactivity the membrane potential was about the same as that recorded between the bursts in the control period.

Figure 1d illustrates the activity of the portal vein in normal Krebs solution 5 min after the period of norepinephrine exposure. The amplitude of the mechanical contractions was still significantly higher than in the control period. After 10 min the pattern of activity was similar to that of the control period.

The effects of a higher concentration of norepinephrine are illustrated in Figure 2. The activity of the portal vein during the control period is seen in Figure 2a. In Figure 2b norepinephrine, 10 mg/liter, was added to the Krebs solution. This resulted in a continuous discharge and slow depolarization during which the action potentials decreased in amplitude and vanished within approximately 1 min. An apparent contracture of 1200 dynes was maintained as long as norepinephrine was present. After 1 min of exposure to norepinephrine, small spikes reappeared (Fig. 2c) but the contracture showed no phasic properties. Three to four minutes after norepinephrine was removed a slow repolarization was recorded (Fig. 2d). Simultaneously the spikes grew in amplitude and the tension became phasic in spite of a high frequency of discharge. After about 10 min in normal Krebs solution (Fig. 2e), the electrical and mechanical activity of the portal vein closely resembled the activity in the control period.

2. In Solution with High [K⁺]. The effect of norepinephrine was studied also on portal vein preparations which were depolarized by replacing all NaCl of the superfusing solution with KCl. A more complete description of the effects of increased [K⁺]₀ is given in the concomitant report (4).

Figure 3 illustrates the activity of a portal vein in normal solution (a) and after 5 min in a solution with 128 mmole K⁺/liter (b). The cells were depolarized, spike activity and phasic contractions were abolished, and there was a maintained contracture of about 400 dynes. Addition of norepinephrine, 1 mg/liter, (at arrow), increased the tension of the contracture by about 200 dynes without detectable change in membrane potential.

These results and those described above for the polarized muscle indicate an effect of nor-
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Response of portal vein to norepinephrine in Ca\(^{2+}\)-free solution. a = Normal solution, b and c = After 1 and 8 min in Ca\(^{2+}\)-free solution, respectively. d = Immediate effect of adding norepinephrine, 10 mg/liter, 10 min after Ca\(^{2+}\) withdrawal. e = First min after return from Ca\(^{2+}\)-free to normal Krebs solution. f = After 5 min recovery. The zero level of the tension scale and the 10 mv marker are as described in the legend to Figure 1.

epinephrine on tension in addition to that mediated by propagated and nonpropagated potential changes.

3. In Ca\(^{2+}\)-free Solution. Elimination of Ca ions from the Krebs solution caused a moderate depolarization and abolished spike discharge and contractile activity of the portal vein as already described (4).

Figure 4a, b, and c illustrate the sequence of events which occurred when the superfusing solution was changed from normal Krebs (a) to Ca\(^{2+}\)-free solution. After a period of frequent bursts of short duration (b), there was complete cessation of electrical and mechanical activity (c). When the muscle had been in Ca\(^{2+}\)-free solution for 10 min, norepinephrine, 10 mg/liter, was added (d). This resulted in reappearance of spikes at relatively high frequency and a tetanus of about 250 dynes. Returning to Ca\(^{2+}\)-free solution without norepinephrine led again to complete quiescence (not shown in the figure). Figure 4e and f illustrates the effects of readmitting Ca\(^{2+}\) by switching to normal Krebs solution.
Control activity was resumed (f) after a transient period of continuous spike discharge characterized by gradually increasing amplitude of spikes and contractions (e).

The ability of norepinephrine (1 to 10 μg/liter) to restore activity in portal vein after activity had ceased in Ca²⁺-free solution varied from one experiment to another. Action potentials could be initiated after more than 30 min of Ca²⁺-free environment, but the amplitude of the spikes tended to decline gradually with time. The force of the contractions was also reduced to a greater extent the longer the muscle had been in Ca²⁺-free solution, and active tension responses were often absent in the recordings after periods of more than 20 min despite clear-cut spike activity.

In 8 experiments ether glycol dinitetraacetic acid (EGTA-0.5 mmole/l) was administered together with Ca²⁺-free solution for various lengths of time. Norepinephrine did not restore activity to these muscles but did so to control preparations which were exposed for comparable time periods to Ca²⁺-free solution without EGTA.

4. Effect of Phenoxybenzamine. Phenoxymethylazone (1 mg/liter) abolished the response to norepinephrine in normal solution as well as in high potassium and Ca²⁺-free solutions. This blocking agent by itself had no apparent influence on the spontaneous activity of the vein when used in the above concentration. In a few experiments an inhibitory effect of norepinephrine was seen after phenoxybenzamine. This inhibitory response pattern was similar to that obtained with isoproterenol and it too was abolished by propranolol as described below.

II. ISOPROTERENOL

A. Effects of isoproterenol concentrations below 1 mg/liter

1. In Normal Krebs Solution. The effects of increasing concentrations of isoproterenol on the spontaneous mechanical activity of the isolated rat portal vein are characterized by a gradual decrease in the force of the individual contractions and by a simultaneous increase in the frequency of the contractions (Fig. 5). This change in the pattern of mechanical activity was seen consistently at isoproterenol concentrations in the range of 1 to 100 μg/liter. A complete absence of contractile activity occurred during the greater part of the exposure period with an isoproterenol concentration of 1 mg/liter in the experiment of Figure 5. An initial "silent" period was sometimes observed also at lower concentrations. The modifications of the response which appeared at the level of 10 mg/liter will be discussed later.

A further analysis of the action of isoproterenol was carried out by simultaneous recordings of mechanical and electrical activity. Figure 6a shows a typical recording obtained.
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**FIGURE 6**
Electrical and mechanical response of portal vein to isoproterenol. a = Control in normal Krebs solution, b = After 2 min in isoproterenol, 100 µg/liter, c and d = After 3 and 40 min recovery in normal solution, respectively. The zero level of the tension scale and the 5 mv marker are as described in the legend to Figure 1.

**FIGURE 7**
Response of depolarized portal vein to isoproterenol. a = Normal Krebs solution, b = Depolarization and contracture produced by increasing [K⁺]₀ to 128 mmole/liter. Isoproterenol, 1 mg/liter, added at arrow. The zero level of the tension scale and the 10 mv marker are as described in the legend to Figure 1.

during a control period, i.e. with the muscle in normal Krebs solution. The spontaneous contractions had a peak amplitude of 1000 to 1100 dynes and the interval between them was about 30 sec. They were distinctly correlated with bursts of spike discharge with a duration of 5 to 6 sec. Isoproterenol, 100 µg/liter, (Fig. 6b) caused a reduction in the level of
the membrane potential between the bursts, a reduction in the burst interval by 50% corresponding to the increased frequency of phasic contractions and a shortening of the duration of each burst. The bursts were reduced even to single or double spikes in several experiments and spike amplitude was frequently diminished. When the complete abolition of contractions occurred as in the bottom record of Figure 5, this corresponded to a complete absence of spike potentials in the sucrose-gap recordings. It is evident, therefore, that the reduction in the force of contractions of the portal vein caused by isoproterenol could to a large extent be ascribed to a change in the pattern of electrical activity (shortening of burst duration). However, a great reduction in the amplitude of the phasic contractions was often observed despite a relatively well sustained spike activity. The discrepancy between the effect on mechanical and electrical activity was sometimes seen in the early recovery period after isoproterenol (Fig. 6c). Here spike discharge and duration of bursts had returned to control while tension development was still greatly reduced. Both electrical and mechanical activity showed full recovery after isoproterenol (Fig. 6d). An example of a typical isoproterenol response with marked reduction in contractile force and relatively well maintained spike activity within the bursts is shown in Figure 10b below.

2. In Solutions with High [K⁺]. Observations like those illustrated in Figure 6 and 10b suggested that the inhibitory effect of isoproterenol on tension development in the portal vein was not entirely due to a reduction in the number or frequency of spikes per burst. To find out whether isoproterenol would be capable of inhibiting the contractile system independently of propagated or nonpropagated potential changes, experiments were performed on portal vein preparations depolarized by high concentrations of K⁺ in the external medium. Figure 7b illustrates a recording obtained when the portal vein was bathed by a Krebs solution in which all sodium chloride had been replaced by potassium chloride. The spontaneous phasic activity, both electrical and mechanical, was abolished in this medium and the muscle maintained a contracture of 450 dynes. Isoproterenol at a concentration of 1 mg/liter (arrow) caused a 50% inhibition of the contracture tension without any measurable change in the potential level. A slow recovery of contracture tension occurred as the drug was rinsed out with high potassium solution (not shown in the figure).

3. Isoproterenol and “Synchronization” in Portal Vein. It is conceivable that an impairment of intercellular conduction in the smooth muscle of the portal vein might contribute to
Effects of different isoproterenol concentrations on portal vein preparation arranged as in Figure 8. Synchronization is maintained despite the changes in frequency and force of contractions. H = hepatic; M = mesenteric.

the reduction in contraction amplitude illustrated in Figures 5 and 6. This aspect may also be of importance with regard to the simultaneously increased frequency of contractions. The entire pattern of response might possibly be classified as a "negative dromotropic action" of isoproterenol; a conduction block could lead to a desynchronization of the preparation with activation of different parts of the muscle by separate and independent pacemakers. Weak contractions occurring at increased frequency would be compatible with such a "functional fragmentation" of the muscle. A special type of experiment was designed to elucidate this possibility.

A side branch of the dissected portal vein was anchored to a metal hook and the ends of the preparation were connected to force-displacement transducers so that the hepatic and the mesenteric parts of the vein were working
at right angles to each other (Fig. 8, sketch). The end portions of the vein were then found to contract synchronously indicating that they were both activated by the same "pacemaker" (Fig. 8A). Only occasionally did we see weak contractions confined to one end of the vein without any response in the other half. That the two recordings were mechanically independent of each other could be shown by pressing each of the transducers so that the respective part of the vein was completely relaxed; this maneuver did not significantly disturb the passive baseline or the active tension development of the other part (Fig. 8B and C). Figure 8D illustrates the effect of a tight ligature around the metal hook and the middle of the vein, applied at the end of the experiment. This ligature produced a conduction block so that the two parts of the vein then started to contract at different rhythms.

The effects of isoproterenol were studied on such "double preparations" of the portal vein. Figure 9 shows recordings obtained in the same experiment as Figure 8 prior to the application of the "blocking" ligature of Figure 8D. Isoproterenol in increasing concentrations reduced the amplitude and increased the frequency of contractions in both parts of the vein, as seen in Figure 9. It is notable that synchronization was well maintained despite the marked changes in contractile activity.

4. Maintenance and Repeatability of Isoproterenol Responses. The mechanical inhibition caused by isoproterenol in concentrations ranging from 1 to 1000 µg/liter were well sustained over short periods of exposure (less
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than 5 min) as seen for instance in Figures 5 and 9 above. When the muscle was exposed to the higher concentrations in this range for longer periods of time, there was a tendency for electrical and mechanical activity to return towards control. The duration of the bursts and the intervals between them increased gradually. A slow recovery of contraction amplitude was also seen. The recovery was more complete the higher the drug concentration and the longer the exposure period. A reversal of the response into a type of reaction similar to that produced by norepinephrine was not observed at isoproterenol concentrations of 100 \( \mu \text{g} / \text{liter} \) or less.

The inhibitory responses to isoproterenol, 1 to 100 \( \mu \text{g} / \text{liter} \), were repeatable when short periods of exposure were used (2 to 5 min). A second administration of isoproterenol was ineffective, however, if given shortly after a previous prolonged exposure which had been associated with return to control activity as described above. This lack of response to the second administration showed that the return to control in the previous period was not caused by decomposition of the drug in the bath fluid but to a decrease in the responsiveness of the muscle to the drug. It was further found that the ability of the muscle to respond with the ordinary inhibitory pattern returned gradually over 15 to 30 min after the previous prolonged exposure.

5. Effect of Propranolol. The electrical and mechanical components of the response to isoproterenol already presented were all abolished when the muscle was exposed to propranolol in concentrations of 1 \( \mu \text{g} / \text{liter} \). Isoproterenol in concentrations below 100 \( \mu \text{g} / \text{liter} \) had virtually no effect on the spontaneous electrical and mechanical activity after propranolol. Propranolol was capable of blocking also the relaxing effect of isoproterenol on the contracture produced by high \( [K^+]_o \). Propranolol itself in these concentrations had no apparent effect on the spontaneous activity of the normally polarized muscle or on the contracture state produced by high \( [K^+]_o \).

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B. Effects of isoproterenol concentrations above 1 \( \text{mg/liter} \)

1. In Normal Krebs Solution. The effects of isoproterenol concentrations in the range of 10 \( \text{mg/liter} \) differed from those presented in the previous sections. Immediately after administration of the drug, there was an inhibitory response similar to that described at lower doses (Fig. 10b), often with an initial complete absence of spike discharge and contractions. The inhibition was not sustained, however, even for short periods of exposure (3 to 5 min) but electrical and mechanical activity recovered and a reversal to an excitatory pattern of response was seen (Fig. 10c). This delayed excitatory reaction resembled in all respects the action of low concentrations of norepinephrine on the portal vein (Fig. 1). A second administration of isoproterenol, 10 \( \text{mg/liter} \), after a short recovery period, gave no initial inhibition but only the excitatory type of reaction which now had an immediate onset (Fig. 10d and e). Only after long recovery periods in drug-free solution (30 to 40 min) could the initial pattern of response be reobtained.

2. In Solutions with High \([K^+]\). The effect of high concentrations of isoproterenol on the contracture tension of the depolarized portal vein was similar to its action on the polarized muscle with regard to maintenance and repeatability of the response. The first exposure to the drug at 10 \( \text{mg/liter} \) was thus characterized by an initial decrease in contracture tension followed within 1 or 2 min by a return and reversal to a level above control. At a second exposure after a short recovery period (less than 30 min), tension development was augmented and the inhibitory phase was lacking.

3. In \( \text{Ca}^{2+} \)-free Solution. The high concentrations of isoproterenol, 10 \( \text{mg/liter} \) were capable of inducing spike discharge and contractile activity in muscles which had been inactivated by elimination of \( \text{Ca}^{2+} \) from the Krebs solution. In this respect the action of high isoproterenol doses resemble the effects of norepinephrine.

4. Effects of Phenoxybenzamine. Phenoxybenzamine in concentrations of 1 \( \text{mg/liter} \)
abolished the excitatory, "norepinephrine-like" responses to isoproterenol at 10 mg/liter in normal as well as in high potassium, and in Ca²⁺-free solutions.

Discussion

The typical and consistent response of the portal vein to norepinephrine was an excitatory one. The electrophysiological changes in the lower range of norepinephrine concentrations (1 to 100 μg/liter) consisted of an increased frequency of discharge, shortening of intervals between bursts and prolonged burst duration. These results are in general agreement with those obtained with microelectrode technique by Funaki and Bohr (5). High concentrations of norepinephrine (10 mg/liter) produced depolarization, and spike potentials became very low and irregular or totally absent from the sucrose-gap recording during part of the exposure period. This may represent a true depolarization below the level of spike generation and conduction or it may reflect desynchronization of electrical activity with initiation of spikes at numerous different foci which may not be recorded by this method. In the latter case the maintained, high level of tension represents a tetanus, due to high-frequent, asynchronous firing, rather than a contracture. Although the main effects of norepinephrine on tension in the portal vein are thus undoubtedly mediated via changes in the pattern of electrical activity, there is evidence that norepinephrine may influence the contractile system independently of propagated or nonpropagated potential changes. Thus, it was frequently noted that low concentrations of norepinephrine increased tension, at unchanged membrane potential, more than could be accounted for by changes in frequency of discharge. Similar observations were done on the rabbit anterior mesenteric vein by Cuthbert and Sutter (6). Su et al. (7) working on pulmonary artery showed contractile responses to norepinephrine in polarized muscle with no change in potential. That tension could be activated in normally polarized striated muscle without potential changes was reported in 1958 by Axelsson and Thesleff (8). Norepinephrine increased the contracture of depolarized portal vein without measurable potential changes. Activation of the contractile mechanism has previously been demonstrated in different types of depolarized muscle (9-11). In the previous article, dissociation of electrical and mechanical activity was shown for polarized portal vein in K⁺-free and Ca²⁺-free solutions (4).

Whatever the mode of activation of the contractile elements, calcium has proved an essential factor. The present experiments with Ca²⁺-free solutions as well as those already presented (4) clearly demonstrate the importance of Ca²⁺ for the spontaneous electrical activity as well as for the activation of the contractile machinery in portal vein. It is of particular interest that norepinephrine was capable of reinitiating spike discharge and contractile response in this muscle after its spontaneous activity was completely abolished in Ca²⁺-free solution. Studies with flame photometry show that the portal vein retains a considerable fraction of its calcium content even after hours in Ca²⁺-free solution (Wahlström, unpublished). This suggests that norepinephrine restores activity either by increasing membrane permeability for extracellular calcium ions which may still be present in sufficient amounts in the recesses of interstitial space or by liberation of bound calcium. Further support for the role of Ca²⁺ is given because EGTA invariably abolished the restoring effect of norepinephrine in Ca²⁺-free solution. Hinke (12) suggested, on the basis of experiments in which he used flow resistance in perfused isolated artery segments as an indicator of vascular smooth muscle "tone," that norepinephrine maintained constriction in a Ca²⁺-free environment by using bound calcium. This conclusion was based on the results of experiments with EDTA and with variations in pH.

Phenoxybenzamine abolished both electrical and mechanical components of the excitatory norepinephrine response in normal solution as well as in high potassium and in Ca²⁺-free solutions. The different response patterns of the portal vein to norepinephrine in differ-
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ent ionic conditions may thus result from a common primary reaction often referred to as "α-receptor stimulation." It is interesting that the mechanism appears to function in quite variable ionic environments and at different membrane potential levels. The effects of "α-receptor stimulation" cannot yet be defined in terms of ionic or metabolic mechanisms. An increased membrane permeability for Ca²⁺ or mobilization of bound calcium appears to be an important factor in vascular smooth muscle, but the norepinephrine effects on membrane potential and spike activity in portal vein suggest that other ions also are involved.

In accordance with the general ranking order of the adrenergic agents with regard to their "affinity" for α- and β-receptors (13), isoproterenol was shown to be considerably less effective than norepinephrine for initiating the excitatory response of the portal vein. The dominating effect of isoproterenol was an inhibitory influence on contractile activity. The characteristics of this response in normal solution were a moderate membrane depolarization, a shortening of the bursts of action potentials, a shortening of the interval between the bursts, and a reduction in tension development that could not be fully accounted for by the change in spike activity. Complete abolition of spikes and contractile activity was sometimes observed. Intercellular conduction in the vein may be impaired by isoproterenol but not to the extent that desynchronization and functional fragmentation of the muscle develop (Figs. 8 and 9).

Isoproterenol inhibition of the contracture tension in depolarized portal vein occurred without measurable changes in the level of the membrane potential. This observation strengthens the impression obtained from the experiments on polarized muscle that inhibition of tension development by isoproterenol cannot be ascribed entirely to its effects on electrical activity.

Both electrical and mechanical components of the inhibitory isoproterenol response in normal solution and the reduction in contracture tension in high [K⁺]o were abolished by propranolol and may, therefore, all be classified as "β-adrenergic effects." The mechanisms by which adrenergic agents inhibit smooth muscle activity have been subjected to extensive investigation. Adrenaline has been shown to relax the smooth muscle of the taenia coli by inhibition of spike activity and hyperpolarization of the cell membrane (14, 15). It was further suggested that these electrophysiological effects of adrenaline could be secondary to the action of the drug on the cell metabolism (16, 17). A metabolic explanation for the inhibition of smooth muscle by adrenergic agents has also been proposed by Mohme-Lundholm (18, 19).

The electrical events that accompany adrenergic inhibition of contractile activity in portal vein are different from those described for taenia coli because the vascular preparation shows depolarization and increased frequency of bursts, each of which encompasses fewer spikes. These differences suggest that the mechanisms involved may not be the same in the two muscles. Bohr (20) has recently suggested, on the basis of experiments with tension recording in isolated strips of coronary vessels, that β-adrenergic inhibition of vascular smooth muscle operates by a mechanism which reduces the concentration of intracellular calcium ions available for contraction. This is of interest with regard to the present observations on portal vein where the inhibitory response to isoproterenol (or to norepinephrine after phenoxybenzamine blockade) resembles in its electrical and mechanical components the activity changes produced by lowering the Ca²⁺ concentration in the medium (compare Figures 5 and 6 above with the effects of Ca²⁺-free solution described in the previous article). Recent experiments by Jenkinson and Morton (21, 22) on depolarized intestinal smooth muscle are of interest in this connection. They found that isoproterenol was more effective than norepinephrine in inhibiting calcium-induced contractures, whereas norepinephrine was more effective in increasing transmembrane potassium fluxes.

The present findings with regard to the maintenance and repeatability of the inhibi-
itory isoproterenol response deserve some comments since the behavior of the muscle in these respects complicated somewhat the analysis of the drug action. A gradually developing reduction in the responsiveness of the inhibitory mechanism to the action of isoproterenol seemed to occur during prolonged or frequently repeated exposures to concentrations of the order of 100 μg/liter. This refractoriness developed faster and persisted longer at higher concentrations and therefore, it also unmasked the excitatory “noradrenaline-like” response to isoproterenol. The sequence of these events was similar in polarized muscle and in the depolarized state. Our observations on the isolated vascular smooth muscle of portal vein are consistent with findings by Butterworth (23) in the intact circulation; he described a reduction and ultimate reversal of the vasodilator and depressor responses to repeated high doses of isoproterenol. Butterworth ascribed his results to a β-adrenergic blocking action of isoproterenol at high doses. The phenomenon must be considered when intra-arterial isoproterenol infusions are used in circulatory experiments with the intention of creating “maximal vasodilatation.”

Acknowledgments
The able technical assistance of Miss Gunilla Eriksson, Mrs. Gun Jidesten and Mr. Lars Stage is gratefully acknowledged.

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doi: 10.1161/01.RES.21.5.619

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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