Neurogenic Contractile Responses of the Circular Smooth Muscle of the Guineapig Vas Deferens

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Abstract—Contractile responses of the circular smooth muscle of the guinea-pig vas deferens to electrical stimulation were recorded isometrically using ring preparations of about 2 mm in breadth. Stimulation of low frequency (2.5 to 10 Hz, 0.05 msec duration) for 20 sec produced twitch contractions which occurred once or repetitively during the application of stimulation. Higher frequency stimulation (20 to 80 Hz) produced biphasic contractions, an initial phasic and a secondary tonic contraction. These contractions were abolished by 3$x$10$^{-6}$ M tetrodotoxin or 3$x$10$^{-5}$ M guanethidine; however, 3$x$10$^{-6}$ M atropine or hexamethonium did not affect the contractions. Prazosin at 10$^{-6}$ M, like 3$x$10$^{-7}$ M yohimbine, increased the amplitudes of twitch contractions to the low frequency stimulation and caused the twitch contractions to occur repetitively. On the other hand, prazosin suppressed the tonic contractions to the high frequency stimulation without substantially inhibiting the phasic contractions. Cocaine at 3$x$10$^{-6}$ M potentiated the twitch contractions in the presence of yohimbine. After in vivo reserpine treatments, the low and high frequency stimulation produced twitch and phasic contractions, respectively; however, tonic contractions were not induced. Prazosin at 10$^{-6}$ M did not qualitatively affect the contractions in preparations from the reserpinetreated animals. These results suggest that the neurogenic contractions of the circular muscle of the guinea-pig vas deferens are sympathetic in nature, but that they are not mediated solely by norepinephrine. Co-release of other transmitters was indicated to occur upon the electrical stimulation of wide frequency range.

Contractile responses of the circular smooth muscle of the guinea-pig vas deferens to electrical stimulation have been scarcely investigated as compared with those of the longitudinal smooth muscle. This may be primarily due to the experimental difficulties in recording the contractions of circular smooth muscle cells that circumferentially line the tubular organ. In the longitudinal muscle of the vas deferens, the neurogenic contractions are mediated not only by norepinephrine but also by another transmitter (1-3). However, such a possibility was not indicated for the circular muscle when the intraluminally perfused preparations, helically cut strips or "Vane" strips were used to record the circular muscle contractions (4, 5). In the present study, ring preparations of the guinea-pig vas deferens have been used in order to directly record the contractions of the circular muscle and also to minimize the artifacts due to the contractions of the longitudinal smooth muscle. The results obtained indicate that neurogenic contractile responses of the circular muscle were not solely attributed to norepinephrine.

Materials and Methods
Male guinea-pigs weighing 350-600 g were stunned and bled to death. Vasa deferentia were dissected out and placed in the petri dish containing the physiological salt solution (PSS) of the following composition (mM): NaCl, 140; KCl, 6.0; CaCl$_2$, 2.0; MgCl$_2$, 1.0; NaH$_2$PO$_4$, 1.0; glucose, 5.5; HEPES (2-[4-(2-hydroxyethyl) piperazin-1-
[...]

yl] ethanesulfonic acid), 5.0, pH 7.3, adjusted by 0.1 N NaOH. Under the stereo-

microscope observation, ring preparations approximately 2 mm in breadth were cut out
from the middle portion of the vas deferens. The weights of ring preparations were be-
tween 2.1 to 5.4 mg when measured after the completion of the experiments. Two “L”
shaped stainless wires were inserted through the lumen of the ring preparation; one end
was fixed to an organ chamber and the other was connected to a force transducer via a
cotton thread. The ring preparations were loaded with the resting tension of 0.3 g and
were allowed to equilibrate for 90 min at 36°C. The organ chamber of 10 ml in volume
was continuously supplied with PSS flowing at the rate of 3 ml/min, and the PSS in the
chamber was bubbled with 100% O₂.

Isometric contractions were measured with a U gage (UL-10, Minebia Co.) and were
displayed on a pen recorder (SR 6211, Graphtec) through an amplifier (6M 91, San-
Ei). Field stimulation was made with an electric stimulator (MSE-3R, Nihon Kohden)
via a pair of platinum electrodes (1 mm x 3 mm) placed on either side of the preparations.
Electrical stimulation was generally applied for 20 sec at various frequencies. Pulse
duration was 0.05 msec in most experiments (0.1 msec in some experiments). The voltage
applied in the closed circuit was approximately 4.4 V.

The frequency-response relationship was determined as follows: After two times of
trial stimulation with 20 Hz, stimulations with increasing frequency (2.5 to 40 or 80 Hz)
were applied with a 10 min interval between the stimulations. In experiments to investigate
the effects of prazosin on the frequency-

response relationship, the same stimulation
procedure was repeated in the presence of prazosin 30 min after the first series of control
experiment was completed. In the test ex-
periments, perfusion with the PSS containing
prazosin was started 10 min prior to the 2nd
series stimulation. A time-matched control
experiment which was done without prazosin
showed that the contractions in the 1st and
2nd series were almost identical except for
those in response to the low frequency
stimulation (Fig. 5A). Unless otherwise
mentioned, the effects of other antagonist
drugs were determined by stopping the
perfusion and adding the drugs to the organ
chamber. The preparations were treated with
the drugs for at least 10 min. Responses were
expressed as the mean±standard errors of the
highest amplitudes of developed tensions.

Reserpine was administered intraperi-
toneally three times, 48, 24 and 0.5 hr before
the experiments with the doses of 5, 2.5 and
2.5 mg/kg, respectively (3).

The drugs used in the present study were:
prazosin HCl (Pfizer), guanethidine sulfate
(Ciba-Geigy), tetrodotoxin (Sigma), atro-
pine sulfate (Sigma), hexamethonium chloride
(Wako), d,l-propranolol HCl (Sumitomo)
reserpine (Daich), yohimbine chloride
(Sigma), cocaine HCl (Takeda), phentol-
amine mesylate (Ciba-Geigy), dibenamine
HCl (Nakarai), 1-norepinephrine (Nakarai),
ATP disodium salt (Sigma), β, γ-methylene
ATP disodium salt (β, γ-mATP, Sigma),
adenosine (Wako), acetylcholine chloride
(Daiichi), and carbachol (Tokyo Kasei).
Norepinephrine and dibenamine were dis-
solved in 0.1 N HCl. All other stock solutions
of drugs were made with distilled water.

Results

Frequency-response relationship: Field
stimulation produced frequency-dependent
contractions in the ring preparations of the
guinea-pig vas deferens. Figure 1A shows
the representative contractions to the elec-
trical stimulation for 20 sec at various
frequencies from 2.5 to 40 Hz (0.05 msec
pulse duration). Although the contractile
responses varied between the preparations
and with time after dissecting tissues, the
relationship between the frequency and con-
tractions was obvious in the individual pre-
parations. Stimuli with low frequency (2.5
or 5 Hz) evoked twitch contractions which
occurred once or repetitively during the ap-
lication of stimulation. As shown in Fig. 1A,
twitch contractions usually developed im-
mediately after the start of stimulation; in
some preparations, the contractions occurred
in the middle or around the end of the
stimulation. Increase of the frequency to 10Hz
produced repetitive twitch contractions of
which magnitudes were initially large and
Circular Muscle of Vas Deferens

Fig. 1. Contractile responses of the circular muscle of the guinea-pig vas deferens to the field stimulation at various frequencies as recorded in the ring preparations. Horizontal bars under the recordings show the period of stimulation applied for 20 sec. Duration of stimulation pulse was 0.05 msec in A and 0.1 msec in B. Similar contractile profile was observed in 10 preparations out 17 (0.05 msec pulse); in the remaining ones, variations such as interruption of twitch contractions (10 Hz) or less developed tonic contractions (20 Hz) were observed. With the 0.1 msec pulse, almost identical contractions were observed in all preparations tested (n=7).

Stimulation with the frequency over 20 Hz produced generally biphasic contractions, initial phasic contractions with rapid rising and then following tonic contractions. Magnitudes of both contractions increased in a frequency-dependent manner. The frequency-response curves were constructed by using the highest peaks of the contractions evoked at each frequency, since the quantitative assessment of the tonic contractions was sometimes difficult as exemplified in the responses to 20 Hz stimulation shown in Fig. 7A. As shown in Fig. 2, the magnitudes of the contractions increased in a frequency-dependent manner.

In the intraluminally perfused preparations, Anstey and Birmingham (4) reported that electrical stimulation of certain frequency and train length consistently evoked an “after-response”, a contraction which occurred after the cessation of stimulation. “After-responses” were also observed in the ring preparations (Fig. 1). However, the occurrence was rare, and no specific conditions seemed to exist to evoke the “after-response” in the ring preparations.

Treatment with $3\times10^{-6}$ M tetrodotoxin then became smaller. Stimulation with the frequency over 20 Hz produced generally biphasic contractions, initial phasic contractions with rapid rising and then following tonic contractions. Magnitudes of both contractions increased in a frequency-dependent manner. The frequency-response curves were constructed by using the highest peaks of the contractions evoked at each frequency, since the quantitative assessment of the tonic contractions was sometimes difficult as exemplified in the responses to 20 Hz stimulation shown in Fig. 7A. As shown in Fig. 2, the magnitudes of the contractions increased in a frequency-dependent manner.

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Treatment with $3\times10^{-6}$ M tetrodotoxin
for 10 min abolished the contractile responses up to 80 Hz (Fig. 3A). Guanethidine at $3 \times 10^{-5}$ M for 20 min almost abolished the tonic contractions and completely inhibited the phasic and tonic contractions after 50 min as shown in Fig. 3B. Atropine or hexamethonium at the concentration of $3 \times 10^{-6}$ M did not affect the contractile responses of the ring preparations to the frequency of 10 and 20 Hz.

With the pulses of 0.1 msec duration, magnitudes of contractions were greater than those for 0.05 msec duration at all frequencies as demonstrated in the frequency-response curves (Fig. 2); tonic contractions started to develop at the lower frequency (10 Hz, see Fig. 1B). “After-responses” were more frequently observed with 0.1 msec pulses (20 and 40 Hz). However, the contractile responses to 0.1 msec pulses were not always abolished by $3 \times 10^{-6}$ M tetrodotoxin or $3 \times 10^{-5}$ M guanethidine as illustrated in Fig. 3. These results indicate that non-neurogenic components may be involved in the responses to the 0.1 msec electrical pulses. In the following experiments, 0.05 msec pulses were used to investigate the characteristics of the neurogenic responses of the circular smooth muscle of the guinea-pig vas deferens.

Effects of drugs affecting the adrenergic mechanisms: Figure 4A shows the effects of an alpha-adrenoceptor antagonist, $10^{-6}$ M prazosin, on the neurogenic contractions, and Fig. 5B illustrates the effects of prazosin on the frequency-response curve. As shown in Fig. 4A, prazosin qualitatively affected the neurogenic contractions. Twitch contractions to low frequency stimulation were repetitively induced after the treatment with prazosin for more than 10 min in most of the preparations. Magnitudes of twitch contractions were

![Diagram](image-url)

**Fig. 3.** Effects of $3 \times 10^{-6}$ M tetrodotoxin (TTX, A) and $3 \times 10^{-5}$ M guanethidine (GUA, B) on the contractions induced by field stimulation with 0.05 msec pulses (upper panel) and 0.1 msec pulses (lower panel). The time shown in the figure represents the exposure period to tetrodotoxin or guanethidine. The frequency used to induce contractions is demonstrated beside the control response. Similar results were observed in 4–7 preparations.
Fig. 4. Effects of $10^{-6}$ M prazosin on the neurogenic contractions at various frequencies in the intact preparations (A, n=6) and reserpine-pretreated preparations (B, n=5). Pulse duration was 0.05 msec for all contractions.

Fig. 5. Effects of $10^{-6}$ M prazosin on the frequency-response curves for the neurogenic contractions in the intact (B) and reserpine-treated preparations (C). To assess the effects of prazosin, two series of stimulation procedures were made in each preparation; the first time in the absence of prazosin (Control) and the second time in the presence of prazosin (see methods for detail). Time-control experiment without prazosin (A) shows that the responses at the low frequency were slightly reduced in the 2nd series stimulation. The numbers in the parentheses are those of the preparations tested. Vertical bars represent standard errors.
also increased (Fig. 5B), although statistical significance was observed only in the response to 5 Hz stimulation (P=0.05, Student's nonpaired t-test). Concerning the contractions to high frequency stimulation over 20 Hz, prazosin suppressed the development of secondary tonic contractions, leaving the phasic contractions almost unaffected. Magnitudes of contractions to high frequency stimulation were not significantly changed by prazosin (Fig. 5B). Effects of $10^{-5}$ M prazosin for 10 min on the responses to the low and high frequency stimulation were similar to those of $10^{-6}$ M prazosin; however, longer treatment with $10^{-5}$ M prazosin inhibited the twitch contractions and slightly reduced the phasic contractions to the low and high frequency stimulation. Like $10^{-6}$ M prazosin, $10^{-6}$ M phentolamine or $10^{-7}$ M dibenamine for 20 min exerted excitatory and inhibitory effects on the responses to the low and high frequency stimulation, respectively (data not shown). Propranolol at $10^{-6}$ M for 15 min did not affect the neurogenic responses at any frequency tested (5–40 Hz).

Three injections of reserpine (5, 2.5 and 2.5 mg/kg, i.p., at 48, 24 and 0.5 hr beforehand) has been reported to reduce the content of endogenous norepinephrine in the guinea-pig vas deferens to 1% of the control (3). Figure 6A shows the actual recordings of contractile responses obtained in such reserpine-treated ring preparations. The frequency-response curve is shown in Fig. 6B. Neurogenic contractions of the reserpine-treated preparations were similar to those obtained in the prazosinized intact preparations. Low frequency stimulation produced repetitive twitch contractions during the stimulation, and high frequency stimulation (more than 20 Hz) produced phasic contractions followed by decaying contractions. However, tonic contractions were never produced by up to 80 Hz stimulation. Application of $10^{-6}$ M prazosin to the reserpine-treated preparations reduced the magnitudes of contractions at all frequencies to some extent (Fig. 5C), although no qualitative changes were induced by prazosin (Fig. 4B). Yohimbine at $3\times10^{-7}$ M raised the responsivity of the ring preparations to the low frequency stimulation (5 and 10 Hz) and increased the amplitudes of twitch contractions (Fig. 7). As observed in the case of prazosin, repetitive twitch contractions were

![Fig. 6](image_url)

**Fig. 6.** Contractile responses of reserpine-pretreated preparations. A, actual recordings to the field stimulation with 0.05 msec pulses at various frequencies. B, frequency-response curve for reserpine-treated preparations (●). For comparison, the frequency-response curve for intact preparations previously shown in Fig. 2 is superimposed (○). The number of preparations used is 7. Vertical bars represent standard errors.
induced by nerve stimulation in the presence of yohimbine. Yohimbine did not potentiate the phasic contractions to 20 or 40 Hz stimulation, but slightly enhanced the tonic contractions.

Effects of $3 \times 10^{-6}$ M cocaine on the responses to 5–20 Hz stimulation were inconsistent among the frequencies: magnitudes of contractions in the absence or presence of cocaine were $0.45 \pm 0.06$ and $0.35 \pm 0.12$ g for 5 Hz, $0.55 \pm 0.07$ and $0.84 \pm 0.09$ g for 10 Hz, and $1.03 \pm 0.11$ and $1.16 \pm 0.11$ g for 20 Hz (mean ± standard error, n=5). However, in the presence of $3 \times 10^{-7}$ M yohimbine, cocaine consistently potentiated the contractions as shown in Fig. 7A, B. In the responses to the low frequency stimulation, a few twitch contractions were usually enhanced. In the responses to 10 Hz stimulation, the tonus was gradually raised after the initial twitch contractions, and then repetitive twitch contractions were superimposed on it. Cocaine did not affect the phasic contractions to the high frequency stimulation, but enhanced the tonic contractions. Magnitudes of tonic contractions for 20 Hz stimulation were increased (Fig. 7). For 40 Hz stimulation, cocaine delayed the onset of relaxation after the termination of stimulation and prolonged the duration of contraction, although cocaine did not increase the magnitudes of tonic contractions (data not shown).

**Role of norepinephrine in the phasic contractions:** Since the phasic contractions to the high frequency stimulation were not considerably affected by adrenolytic drugs such as prazosin and reserpine or by cocaine, there seemed to be little involvement of norepinephrine in the phasic contractions. However, the supporting effects of norepinephrine in the development of phasic contractions were demonstrated when phasic contractions were repeatedly generated by the recurrent stimulations by short length trains. Figure 8 shows that in the control preparations, phasic contractions of almost identical magnitude were repetitively induced.
Fig. 8. Effects of $10^{-6}$ M prazosin (A) and $3 \times 10^{-7}$ M yohimbine (B) on the phasic contractions repeatedly induced by recurrent stimulation by short trains (2 sec, 20 Hz) at an interval of 3 sec. Trains of stimulation applied were depicted as the broken line under the recordings. Shown in the left side are the recordings to the stimulation of 20 sec period (20 Hz). Similar results were observed in 4 preparations out of 4 for prazosin and yohimbine.

by the short train stimulation of 2 sec (20 Hz) with an interval period of 3 sec between the stimulations. However, after the treatment with $10^{-6}$ M prazosin, the magnitudes of phasic contractions decayed rapidly after the initial few stimulations and remained at the lower level thereafter (Fig. 8A, 4 preparations out of 4). The regenerative development of the phasic contractions were not affected by $3 \times 10^{-7}$ M yohimbine (Fig. 8B, 4 preparations).

Contractions to exogenous drugs: Figure 9A shows the typical contractile response of the ring preparations to $10^{-3}$ M norepinephrine, ATP and carbachol. In general, contractions of ring preparations to agonist drugs were variable among the preparations. The threshold concentration of norepinephrine was relatively high and subject to considerable variation, ranging from $3 \times 10^{-6}$ to $10^{-4}$ M. As shown in Fig. 9A, there was always a lag time before the contractions occurred in response to norepinephrine. The contractions to high concentration of norepinephrine consisted of phasic contractions recurring in the presence of norepinephrine. Low concentrations of norepinephrine produced repetitive twitch contractions. The dose-response curve to norepinephrine is shown in Fig. 9B. Cocaine of $3 \times 10^{-6}$ M shifted the dose-response curve to the left by about 3-fold.

ATP at the concentrations of more than $10^{-4}$ M evoked contractions with rapid onset. The magnitudes and duration of contractions increased in a dose-dependent manner. $\beta,\tau$-mATP, an ATP analogue which is less susceptible to hydrolysis, evoked contractions similar to those by ATP. The dose-response curve to ATP and $\beta,\tau$-mATP are shown in Fig. 9C. $\beta,\tau$-mATP was approximately 100-fold more potent than ATP. Contractions in response to ATP and $\beta,\tau$-mATP were not affected by $10^{-6}$ M prazosin. Adenosine at $10^{-3}$ M did not cause any contractions.

Carbachol at $10^{-4}$–$10^{-3}$ M induced contractions in the ring preparations. The tonus slowly arose upon the addition of carbachol, and then twitch contractions were repetitively induced on the elevated tonus (Fig. 9A). Similar contractions were induced by $10^{-3}$ M acetylcholine. The contractions to carbachol or acetylcholine were abolished by $10^{-6}$ M atropine, but were not affected by $10^{-6}$ M prazosin.

Discussion

As far as we surveyed the references, the present study is the first one which uses circular ring preparations to record the contractile responses of the circular smooth muscle of the guinea-pig vas deferens. Previously, with the intraluminally perfused preparations, Anstey and Birmingham investigated the contractile responses of this circular smooth muscle to field stimulation (4, 5). However, the change in the perfusion pressure is an indirect expression of the contractions of the circular muscle. In the present study, contractile responses were recorded directly from the circular muscle using ring preparations where artifacts deriving from the contractions of the longitudinal muscle could be minimized. Therefore, the results obtained in the
Fig. 9. Contractions of the circular muscle of the guinea-pig vas deferens to exogenous drugs. A, actual recordings to 10^{-2} M norepinephrine (NE), ATP and carbachol (CCH). At the black dots, the agonist drugs were applied and present thereafter. B, dose-response curves for the contractions to norepinephrine (NE) before and after the treatment with 3 \times 10^{-6} M cocaine (COC). The number of preparations used was 5. C, dose-response curves to ATP and \( \beta \),\( \gamma \)-methylene ATP (mATP). The number of preparations was 9 for each curve. Abscisae in B and C are the concentrations of agonist drugs expressed as the negative logarithm of molar concentrations (–log M). Vertical bars represent standard errors.

The main finding of the present study is the fact that neurogenic contractions of circular muscle of the guinea-pig vas deferens had a component which was resistant to adrenolytic drugs. The tonic contraction in response to high frequency stimulations may be induced by norepinephrine since this component was suppressed by \( \alpha \)-blockers or reserpine-pretreatment. However, twitch and phasic contractions to the low and high frequency stimulation respectively persisted in the presence of \( \alpha \)-blockers in the intact preparations. Reserpine-treated preparations exhibited contractions similar to those obtained in the prazosin-treated intact preparations, and such contractile responses were not affected by prazosin. These results suggest that a transmitter other than norepinephrine may be substantially involved in the neurogenic contractions of the circular smooth muscle. In various smooth muscles, it has recently been reported that the neurogenic contractions are not solely ascribed to the classical transmitters such as norepinephrine or acetylcholine (6–8). The release of another transmitter responsible for the non-adrenergic and non-cholinergic contractions has been postulated in these smooth muscles. In the longitudinal muscle of the guinea-pig vas deferens, accumulating evidence suggests that ATP is such a transmitter and that norepinephrine and ATP which are co-transmitted from the sympathetic nerves may respectively contribute to the tonic and phasic component of the contractions elicited by the nerve stimulation with a proper frequency (1–3, 9). Most of the current results may well be explained by assuming that a similar mechanism is operative in the
circular smooth muscle, although it is not known from the present study whether or not the hypothetical transmitter is ATP in the circular muscle.

In the longitudinal muscle, either low frequency stimulation or high frequency stimulation has been reported to induce phasic contractions (9), indicating that actions of the putative transmitter, i.e., ATP, are similar in property irrespective of the frequency. On the other hand, in the circular muscle, actions of the unknown transmitter may differently appear depending on the stimulation frequency. With the low frequency stimulation, such a transmitter may elicit repetitive twitch contractions, and with the higher frequency stimulations, the phasic contractions may be induced. Two explanations could be considered for this change in the actions of the unknown transmitter. Firstly, the release of the putative transmitter may cease rapidly due to accommodation or other depression in the neuronal function during the continuous stimulation with high frequency. This explanation, however, appears implausible in view of the fact that the hypothetical transmitter and norepinephrine are co-released from the sympathetic nerves. Since norepinephrine release is thought to continue during the period of stimulation as indicated by the development of tonic contraction in the intact preparations, it is hard to suppose that the release of the co-transmitter is suppressed. Alternatively, despite the continuous release of the transmitter, contractions may decline rapidly due to the desensitization of the smooth muscle to the transmitter in response to the high frequency stimulations. At present, this explanation is difficult to ascertain because of the paucity of knowledge about the responsible transmitter. However, rapid desensitization to the released transmitter has been indicated in the rabbit ear artery and longitudinal muscle of the guinea-pig vas deferens (1, 7).

Norepinephrine may mainly contribute to the tonic contractions to the high frequency stimulations. In addition to this, the present results indicate that norepinephrine may have modulatory effects on the twitch and phasic contractions. The results that yohimbine potentiated the twitch contractions suggest that norepinephrine released in response to low frequency stimulation acts predominantly on the presynaptic \( \alpha \)-receptors, leading to the inhibition of the release of transmitters. When the presynaptic \( \alpha \)-receptors and the uptake mechanism were inhibited by yohimbine and cocaine, respectively, the postsynaptic excitatory action of norepinephrine may be manifested in response to the low frequency stimulation. The enhancement of twitch contractions by prazosin may be explained if prazosin blocked the presynaptic \( \alpha \)-receptors to some extent, as suggested in the longitudinal muscle of the guinea-pig vas deferens (1).

Concerning the modulatory action of norepinephrine on the phasic contractions, it seems worthwhile to point out that phasic contractions could be regeneratively induced by a short train of stimulations when the action of released norepinephrine was not impaired, whereas in the blockade of \( \alpha \)-receptors, the phasic contractions decayed with a few repetitions of stimulation. These results suggest that norepinephrine may have a supporting effect for the development of repetitive phasic contractions, although norepinephrine may not be essential for the generation of phasic contractions. This effect of norepinephrine would be meaningful for the repetitive development of phasic contractions in the physiological state where it has been shown that impulses of neurons occurred intermittently in a burst (10).

The circular muscle of the guinea-pig vas deferens responded to norepinephrine, ATP and acetylcholine. The slow onset of contractions to exogenous norepinephrine is consistent with the delayed action of endogenous norepinephrine. The observation that ATP and \( \beta,\gamma \)-mATP produced contractions that rise rapidly may be indicative that ATP is the co-transmitter in the circular smooth muscle of the guinea-pig vas deferens. Although acetylcholine and carbachol induced repetitive twitch contractions, acetylcholine may be excluded as the transmitter since atropine did not affect the neurogenic contractions.

In conclusion, the present results showed that the ring preparations were appropriate
for recording the contractile responses of the circular smooth muscle of the guinea-pig vas deferens. The neurogenic responses of this smooth muscle were sympathetic in nature, but were not solely due to norepinephrine. It was suggested that another transmitter was involved and that the transmitter and norepinephrine were co-released upon the electrical stimulation of wide frequency range. Identification of the supposed transmitter awaits further study.

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