Further Evidences for the Cholinergic and Adrenergic Mechanisms in the Isolated Hypogastric Nerve-Seminal Vesicle Preparation of the Guinea-Pig

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INTRODUCTION

When hypogastric nerve was stimulated, the contractile response is potentiated by adrenaline or noradrenaline and the response is reduced by adrenergic blocking drugs (Guimaraes[1], Saxena[2]). The excitatoıy junction potentials from the smooth muscle of the guinea-pig seminal vesicle are also inhibited by adrenergic agents (Takeda et al)[3]. These results suggest adrenergic innervation to the smooth muscle of seminal vesicle. Furthermore, histochemical studies indicate postganglionic sympathetic innervation of seminal vesicles (Sjöstrand[4], Wakeda et al[5]). It is also demonstrated histologically (Falck et al[6]).

On the other hand, it had been reported that acetylcholine produced a vigorous contraction of the guinea-pig seminal vesicle (Spedding et al)[7]. Also, cholinergic blocking agents alter the contractile responses to nerve stimulation (Sexena)[2]. Recently some authers emphasized the existence of independent cholinergic and adrenergic mechanisms to the musculature of the guinea-pig vas deferens (Bell[8]; Bharagava et al[9]).

The present experiment was undertaken to study the adrenergic and cholinergic mechanisms in the guinea-pig isolated hypogastric nerve-seminal vesicle preparation.

METHODS

The whole seminal vesicle of the guinea-pig with attached hypogastric nerve was prepared in a manner similar to that described by Naimzada[10]. The distal end of each vesicle was cut through and some of the seminal fluid gently expelled. The experimental methods were the same as described in the previous paper (Ohkawa)[11]. When the seminal vesicle was stimulated transmurally, an electrode similar to the one employed by Birmingham et al[12] was used. The stimulator (MSE-40, Nihon kohden) was set to deliver pulses of 0.01–0.05 msec duration at 10–20 pulses/sec for 5 sec. These high frequencies were used for the transmural

or hypogastric nerve stimulation. The hypogastric nerve trunk was stimulated at a distance of 4–5 cm from the seminal vesicle. The low frequency (1–5 pulses/sec) was also used to count the required pulses for producing the contraction. For the direct stimulation to the tissue, wide single pulse (10 msec or more in duration) was used.

The following drugs dissolved in saline, were employed; acetylcholine chloride, adrenaline hydrochloride, atropine sulfate, bevonium methylsulfate, carbachol, guanethidine sulfate, hexamethonium bromide, metachloine chloride, neostigmine bromide, nialamide, nicotine, noradrenaline bitartrate, phenoxybenzamine hydrochloride, phenylephrine hydrochloride, physostigmine sulfate, tetrodotoxin (TTX) and xylocaine.

RESULTS

1. Mechanical activity of the isolated seminal vesicle

The preparation usually showed no spontaneous contractions. When the stimulation was given to hypogastric nerve, transmurally or directly, a phasic contraction was produced. After 10–30 min exposure to the normal solution, spontaneous mechanical activity was observed in about 30 % of the preparations. The patterns of the spontaneous contractile activity were different from preparation to preparation. Some of them exhibited the rhythmic contraction with an interval of 10–20 sec.

Stimulation of the hypogastric nerve produced a strong, phasic contraction. The magnitude and duration of contraction for a stimulus of given characteristics are variable (Fig. 1). For excepting a direct effect of stimulation to smooth muscle of the segment, short pulse, less than 0.05 msec, and weak voltage was used. The reproducible responses produced by the hypogastric nerve stimulation were completely blocked by TTX (1.6×10^{-7}g/ml), so that it is considered that such stimulation is effective to the nervous elements only. The transmural stimulation also produced a reproducible phasic contraction of which height and duration were depended on given stimulations. The responses by transmural stimulation were also blocked by TTX 1.6×10^{-7}g/ml.

Hexamethonium, added to the stimulated preparation in concentration from 10^{-6} to 10^{-5}g/ml, blocked the responses to hypogastric stimulation leaving the contractions due to transmural stimulation. Nicotine (10^{-6} to 10^{-4}g/ml) also reduced and finally blocked the responses to hypogastric nerve stimulation without reducing the responses to transmural stimulation. TTX (1.6×10^{-7}g/ml) blocked the responses to nerve and transmural stimulation. However the effect of direct stimulation was not reduced in TTX. Xylocaine (10^{-5}g/ml) blocked the responses obtained by nerve stimulations. Spontaneous contractions were not abolished in hexamethonium, nicotine or TTX. Fig. 2 shows the spontaneous contraction in various drugs.

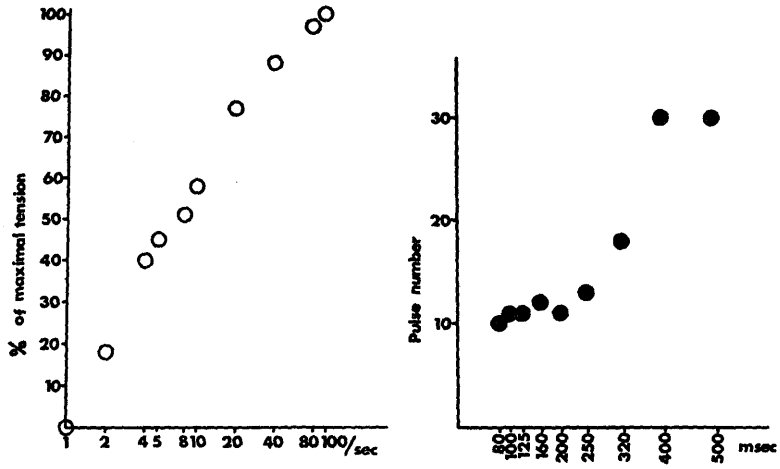

Fig. 1. Left; Relation between the frequency of the transmural stimulation and tension development of the seminal vesicle preparation. The stimulation was 0.05 msec for 5 sec. Right; Relation between the pulse number for producing a contraction and the pulse interval.

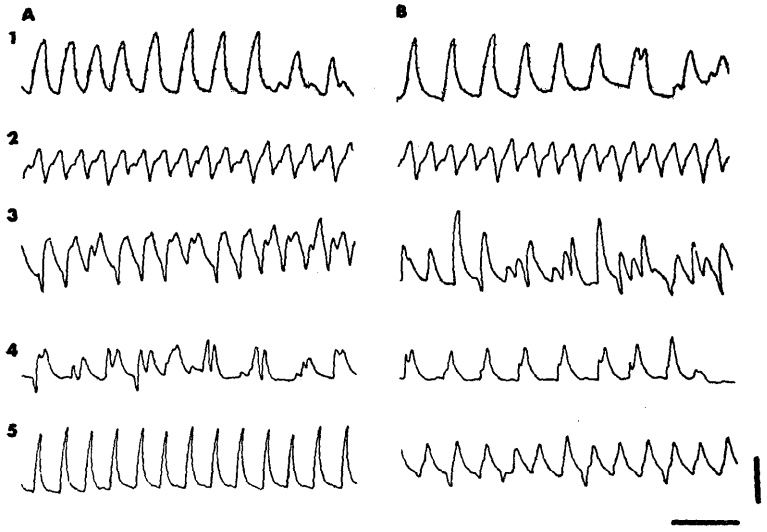

Fig. 2. Spontaneous activity of the isolated seminal vesicle and the effects of drugs.
A (1-5); control, B1; TTX 1.6×10^{-7}g/ml, B2; hexamethonium 10^{-5}g/ml, B3; nicotine 10^{-5}g/ml, B4; guanethidine 10^{-5}g/ml and B5; atropine 1.7×10^{-6}g/ml. Calibration, 30 sec and 0.2g.

2. Effects of drugs related with adrenergic mechanism

Adrenaline (10^{-6} to 3×10^{-6} g/ml), noradrenaline (10^{-8} to 10^{-6} g/ml) or phenylephrine (3×10^{-6} to 10^{-5}g/ml) enhanced the responses produced by the stimulation of hypogastric nerve and by the transmural stimulation. In high concentration of noradrenaline (10^{-6}g/ml), the spontaneous activity was elicited or increased. In the present experiment, a potentiation of the responses to nerve stimulation or transmural stimulation was seen with 10^{-6} to 10^{-4}g/ml concentrations of nialamide, monoamine oxidase inhibitor (Fig. 4). Guanethidine at a concentration of 10^{-5} to 10^{-4}g/ml reduced the responses to hypogastric nerve or transmural stimulation. Slight reduction in the responses to hypogastric nerve stimulation was observed with 3×10^{-6} to 10^{-5}g/ml concentrations of phenoxybenzamine. Direct stimulation potentiated the response in some preparations. Fig. 3 shows the effects of adrenaline and bevonium on the responses produced by stimulations.

3. Effects of drugs related with cholinergic mechanism

Acetylcholine (10^{-8}g/ml) increased the responses produced by direct, transmural and hypogastric nerve stimulation. Spontaneous contraction were also increased by acetylcholine 10^{-8}g/ml. When acetylcholine (10^{-7} to 10^{-6}g/ml) was applied, a strong contraction was produced initially but this contraction was reduced gradually to near the original level or slightly above it. After 3–5 min, the responses to direct stimulations were slightly reduced in acetylcholine 10^{-7}g/ml. Carbachol (10^{-7} to 10^{-5}g/ml) and metacholine (10^{-7} to 10^{-6}g/ml) potentiated the responses of the seminal vesicle to transmural stimulation. Higher dose of carbachol produced spontaneous phasic contractions or contracture. Atropine (10^{-7} to 1.7×10^{-6}g/ml) and bevonium (3.4×10^{-5}g/ml) always caused a reduction in the amplitude of contraction by hypogastric nerve stimulation. The responses to transmural stimulation were not abolished by atropine or bevonium. In the presence of cholinergically acting agents, physostigmine (10^{-7} to 10^{-6}g/ml) and neostigmine (10^{-6} to 10^{-5}g/ml), a potentiation of the responses by direct, transmural and hypogastric nerve stimulation could be observed (Fig. 7).

4. Combined effects of drugs related with adrenergic and cholinergic mechanisms

After the responses initiated by direct, transmural and hypogastric nerve stimulation were potentiated in adrenaline (3×10^{-6}g/ml), bevonium (1.7×10^{-5} to 3.4×10^{-5}g/ml) inhibited the responses produced by transmural and hypogastric nerve stimulation but the response produced by direct stimulation was not inhibited. After guanethidine (10^{-5} to 10^{-4}g/ml), acetylcholine (10^{-7}g/ml) enhanced the responses produced by hypogastric nerve and transmural stimulation. Phenoxybenzamine (1.5×10^{-6}g/ml) reduced the responses by hypogastric nerve and transmural stimulation. After phenoxybenzamine, acetylcholine 10^{-7}g/ml potentiated both responses and the responses were reduced by further addition of atropine 10^{-6}g/ml. Fig. 5 and 6 show some combined effects of drugs on the responses by transmural and hypogastric nerve stimulation.

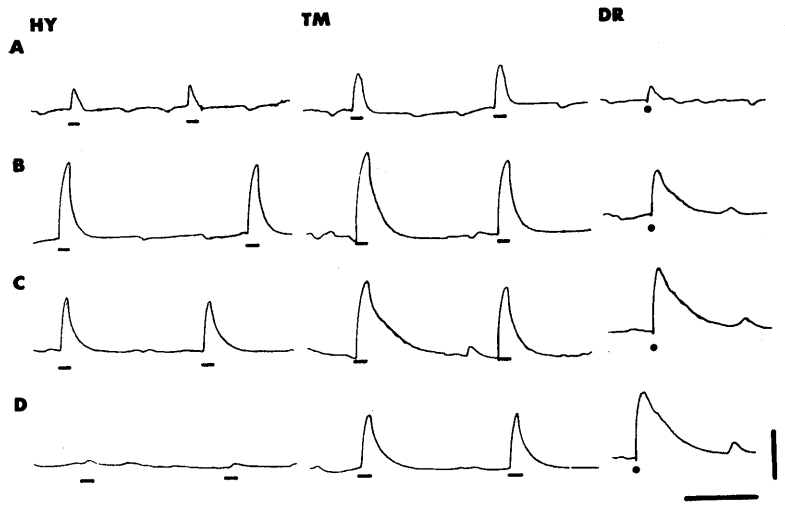

Fig. 3. Effects of adrenaline and bevonium on the responses produced by hypogastric nerve (HY) and transmural (TM) stimulation. A; control, B; adrenaline 3×10^{-6}g/ml, C; adrenaline 3×10^{-6}g/ml + bevonium 1.7×10^{-5}g/ml, D; adrenaline 3×10^{-6}g/ml + bevonium 3.4×10^{-5}g/ml. HY (0.05 msec, 20/sec) and TM (0.01 msec, 20/sec) were applied at the under bar for 5 sec. Direct stimulation (DR; 50 msec, single pulse) was given at the dots. Calibration, 30 sec and 0.2g.

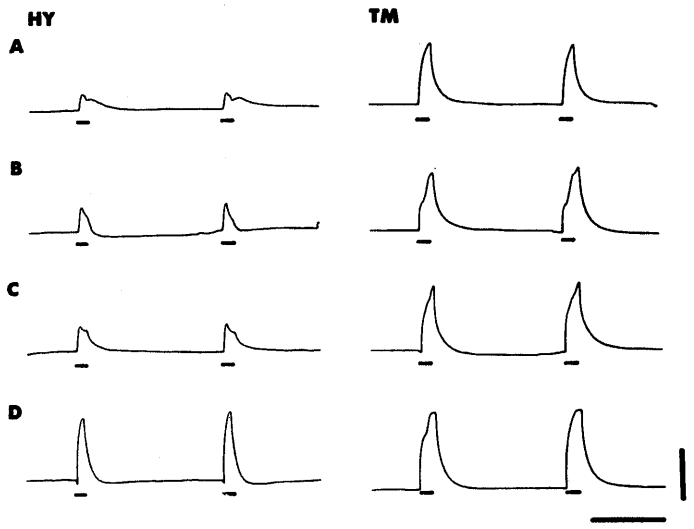

Fig. 4. Effects of nialamide on the responses produced by hypogastric nerve (HY) and transmural (TM) stimulation. A; control, B; nialamide 10^{-6}g/ml, C; nialamide 10^{-5}g/ml, D; nialamide 10^{-4}g/ml. HY (0.05 msec, 20/sec) and TM (0.01 msec, 20/sec) were applied at the under bar for 5 sec. Calibration, 30 sec and 0.2g.

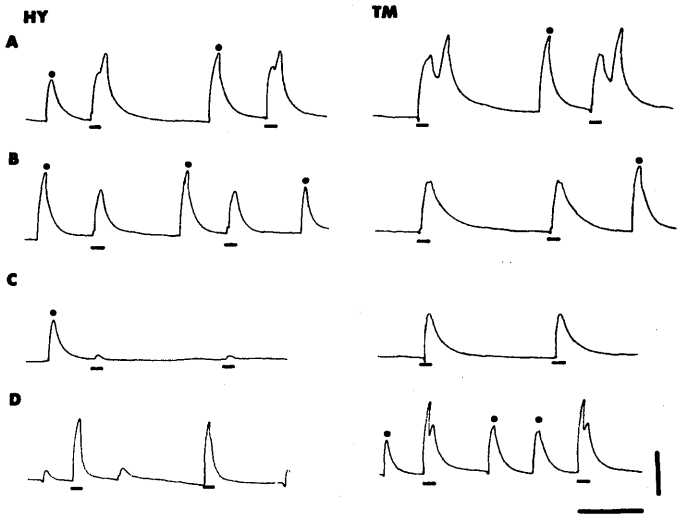

Fig. 5. Effects of guanethidine and acetylcholine on the responses produced by hypogastric nerve (HY) and transmural (TM) stimulation. A; control, B; guanethidine 10^{-5}g/ml, C; guanethidine 10^{-4}g/ml, D; guanethidine 10^{-4}g/ml + acetylcholine 10^{-7}g/ml. HY (0.05 msec, 20/sec) and TM (0.05 msec, 20/sec) were given at the under bar for 5 sec. The black dots in the figure indicate the spontaneous phasic contractions. Calibration, 30 sec and 0.2g.

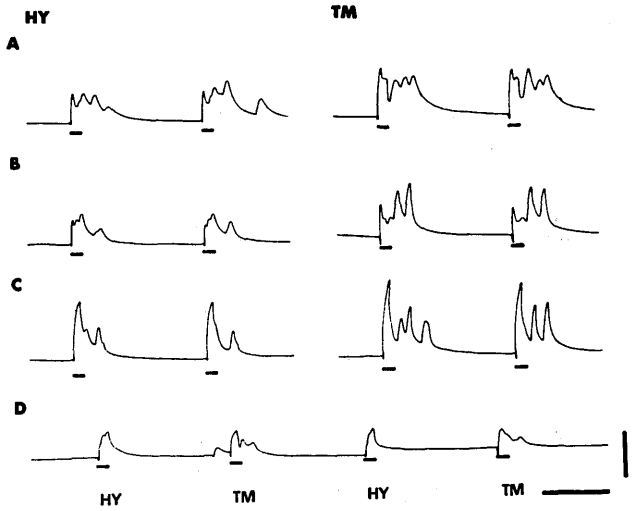

Fig. 6. Effects of phenoxybenzamine, acetylcholine and atropine on the responses produced by hypogastric nerve (HY) and transmural (TM) stimulation.
A; control, B; phenoxybenzamine 1.5×10^{-6} g/ml, C; phenoxybenzamine 1.5×10^{-6}g/ml + acetylcholine 10^{-6}g/ml, D; further addition of atropine 10^{-6}g/ml to the organ bath. HY (0.05 msec, 20/sec) and TM (0.05 msec, 20/sec) were given at the under bar for 5 sec. Calibration, 30 sec and 0.2g.

5. Effects of transmural stimulation in various drugs

Burnstock et al[13] reported that repetitive stimulation of the hypogastric nerve post-ganglionically at 1 pulse/sec produced excitatory junction potentials (EJPs). Raising the voltage of nerve stimulation caused an increase in the amplitude of the EJPs and elicited an action potential (Bell)[8]. This caused contraction of the guinea-pig vas deferens (Bell)[8].

In the present experiment, the hypogastric nerve was stimulated post-ganglionically at 1-5 pulses/sec. When the voltage of stimulation was weak, a response was obtained after several pulses. Numbers of the pulses needed to produce the response depended on the voltage of stimulation. The number of pulses was decreased with increase in voltage.

The required pulse number was decreased in the following drugs; nialamide (10^{-5} to 3×10^{-5}g/ml), metacholine (10^{-7} to 10^{-6}g/ml), physostigmine (10^{-7} to 10^{-6}g/ml), neostigmine (10^{-6}g/ml) and carbachol (10^{-7}g/ml). In phenoxybenzamine (10^{-5}g/ml), atropine (10^{-7} to 10^{-6}g/ml) and guanethidine (10^{-5}g/ml), the pulse was increased. The obtained results were summarized in Fig. 8 and Table 1.

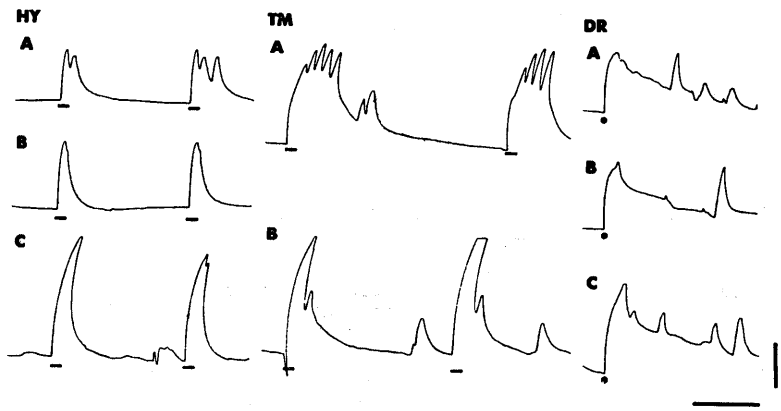

Fig. 7. Effects of physostigmine on the responses produced by hypogastric nerve (HY), transmural (TM) and direct (DR) stimulation. HY and DR; A: control, B: physostigmine 10^{-6}g/ml, C: physostigmine 10^{-5}g/ml. TM: A: control, B: physostigmine 10^{-5}g/ml. HY (0.05 msec, 20/sec) and TM (0.01 msec, 20/sec) were given at the under bar for 5 sec. DR (10 msec) was given at the dots. Calibration, 30 sec and 0.2g.

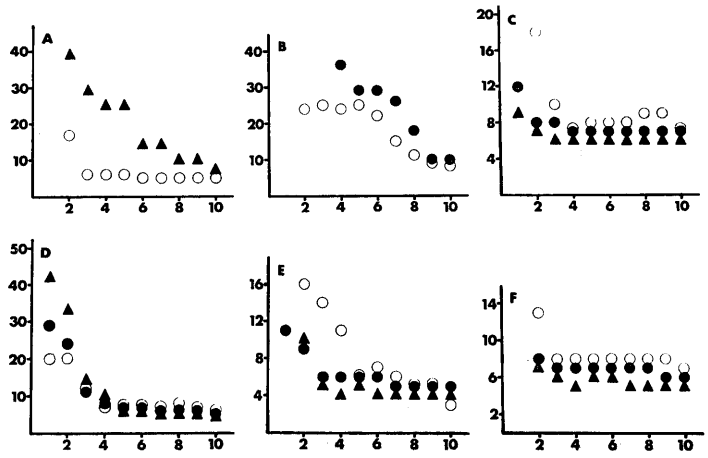

Fig. 8. Effects of various drugs on the pulse number for producing the response by transmural stimulation. White circles in A-F; control, Horizontal; relative intensity of the stimulation. Ordinate; pulse number. A; guanethidine 10^{-5}g/ml (▲), B; phenoxybenzamine 10^{-5}g/ml (●), C; nialamide 10^{-5}g/ml (●) and 3×10^{-5}g/ml (▲). D; atropine 10^{-7}g/ml (●) and 10^{-6}g/ml (▲). E; neostigmine 10^{-6}g/ml (●) and carbachol 10^{-7}g/ml (▲). F; metacholine 10^{-7}g/ml (●) and 10^{-6}g/ml (▲).

Table I. Effects of various drugs on the required pulse number for producing a contraction by transmural stimulation.

Solutions	N	Pulse number for producing a contraction (%)	p value difference
Control	8	100.0±5.4	
Atropine 10^{-6}g/ml	5	111.7±2.3	<0.05
Control	6	100.0±12.9	
Physostigmine 10^{-7}g/ml	6	83.2±18.0	<0.01
Physostigmine 10^{-6}g/ml	7	61.2±20.5	<0.01
Control	10	100.0±13.4	
Metacholine 10^{-7}g/ml	10	55.4±13.4	<0.01
Metacholine 10^{-7}g/ml + Atropine 10^{-6}g/ml	10	103.6±12.0	

DISCUSSION

The effects of hypogastric nerve stimulation and transmural stimulation were abolished by TTX while the direct stimulation was not reduced by TTX. It is also reported that action potentials of taenia coli smooth muscle were not blocked by TTX (Kuriyama et al)[14]. The results of TTX on smooth muscle may indicate that both stimulations are effective only to the nervous factors in the preparation. Therefore the results that the abolition of the response produced by hypogastric nerve stimulation and the remains of the response by transmural stimulation in hexamethonium or nicotine indicate that the existence of ganglionic synapse in the hypogastric nerve near the organ. In the guinea-pig vas deferens, histochemical study also suggested that the presence of a ganglion in the hypogastric nerve near the organ (Wakeda et al)[4].

The spontaneous contractile activity of the seminal vesicle preparation continued in TTX, hexamethonium, guanethidine, phenoxybenzamine and atropine. This mechanical activity was potentiated by Ba^{++} and inhibited by Mn^{++} (Ohkawa, unpublished data). From above results, the activity may due to the spontaneity of smooth muscles of the seminal vesicle preparation.

The effects of drugs related to adrenergic mechanism confirmed with the results obtained with seminal vesicle (Saxena[2], Naimzada[10]) and vas deferens (Bhargava et al[9], Bentley[15], Ohlin et al[16], Hukovic[17], Birmingham et al[12], Sjöstrand[4], Birmingham[18], Holman et al[19], Bella et al[20], Large[21]). That is, the responses by transmural and hypogastric nerve stimulation were potentiated by catecholamine and inhibited by adrenergic ganglion blocking agent and adrenergic inhibitors.

When Naialamide, MAO inhibitor, was applied, the responses produced by transmural and hypogastric nerve stimulation were enhanced. The result was confirmed with the obtained results with pheniprazine and tranylcypromine, MAO inhibitors, in the guinea-pig vas deferens (Bhargava et al)[9]. From above results, it is considered that the seminal vesicle smooth muscles are innervated by α-excitatory post-ganglionic sympathetic nerve.

Naimzada[10] obtained negative results on the cholinergic mechanism in adrenergic fibers of the guinea-pig seminal vesicle preparation while Saxena[2] suggested the existence of a cholinergic mechanism in seminal vesicle. Recently Bhargava et al[9] and Bell[8] bring the presence of cholinergic fibers in sympathetic nerves on the guinea-pig vas deferens. In the present experiment, the cholinergic mechanism in the seminal vesicle preparation was examined by using drugs related with the mechanism.

Acetylcholine, metacholine and carbachol potentiated the responses produced by transmural stimulation. The spontaneous contractile activity was elicited or potentiated by above agents. In higher concentration of acetylcholine, the responses to direct stimulation were reduced. However, this diminution may due to

decrease in the membrane potential of smooth muscle in acetylcholine. The responses were inhibited or potentiated by cholinergic blocking agents or cholinesterase inhibitors respectively.

In the present experiment, the required pulse number for producing the same contraction was examined in various agents. If a single pulse is effective, the EJP may produce by each pulse and an action potential and a phasic contraction may be produced as the result of fascilitation of EJPs. In normal solution, under the condition of same frequency of stimulation, the pulse number was reduced with increasing the pulse strength and vice versa. The required pulse number was increased by adrenergic blocking drugs and reduced by MAO inhibitor. The results also indicate the α-excitatory sympathetic innervation to seminal vesicle smooth muscles.

On the other hand, the required pulse number was reduced by cholinesterase inhibitors and cholinester but increased by muscarinic blocking agent.

The evidence obtained to support the concept of an intermediary cholinergic mechanism in adrenergic fibers has indicated that this system is resistant to muscarinic blocking agents, and sensitive to nicotinic blocking agents (Burn et al)[22)23)]. However, in the present experiment, it has been demonstrated that the muscarinic blocking agent atropine, in low concentrations (10^{-7} to 10^{-6}g/ml), reduces the required pulses. The response produced by transmural stimulation was observed in hexamethonium and the spontaneous contraction was enhanced or elicited by acetylcholine and metacholine. The above results suggest that EJPs in the seminal vesicle smooth muscle are affected by atropine, also physostigmine, and that the presence of acetylcholine receptor in the surface membrane of seminal vesicle smooth muscle.

It is possible to conclude from the obtained results that separate cholinergic and adrenergic motor fibers serve the smooth muscles of the guinea-pig seminal vesicle.

SUMMARY

Effects of autonomic agents on the responses produced by hypogastric nerve and transmural stimulation of the hypogastric nerve-seminal vesicle preparation of the guinea-pig were examined. Hexamethonium and nicotine blocked the responses to hypogastric stimulation leaving the contractions due to transmural stimulation. TTX and xylocaine blocked both responses. Spontaneous contraction which was observed in 30 % of the preparations was not abolished by TTX, hexamethonium, guanethidine and atropine. Acetylcholine, metacholine and noradrenaline elicited or potentiated the spontaneous activity.

Adrenaline, noradrenaline, phenylephrine and nialamide enhanced the responses

produced by the stimulation of hypogastric and transmural stimulation while guanethidine and phenoxybenzamine reduced the responses. Acetylcholine, metacholine, carbachol and cholinesterase inhibitors increased the responses. Atropine and bevonium inhibited the responses. Required pulse number in the transmural stimulation for producing the contraction was decreased in nialamide, metacholine, carbachol, physostigmine and neostigmine, and increased in phenoxybenzamine, guanethidine and atropine.

The possibility that adrenergic and cholinergic mechanisms operate independently of each other in the seminal vesicle preparation was discussed.

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