RESISTANCE OF MECHANICAL AND ELECTRICAL RESPONSES TO HYPOGASTRIC NERVE STIMULATION TO α-BLOCKING AGENTS IN GUINEA-PIG VAS DEFERENS

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In spite of the availability of much evidence showing the sympathetic nature of the hypogastric nerve innervating the guinea-pig vas deferens (1-8) a persistent resistance of the contractile response to hypogastric nerve stimulation to various adrenergic α-blocking agents in vitro has still remained unexplained.

Boyd et al. (9) and Ohlin and Strömback (10) observed that the contractile response to hypogastric nerve stimulation was not depressed by moderate doses of various α-blocking agents but rather potentiated owing to their anti-cholinesterase activity. Burnstock and Holman (8) have also demonstrated that the junction potentials in response to hypogastric nerve stimulation had a considerable resistance to the α-blocking agents in the guinea-pig vas deferens in vitro.

The present study was, therefore, designed to reappraise the well known in vitro resistancy, of mechanical and electrical responses of guinea-pig vas deferens to hypogastric nerve stimulation to α-blocking agents, using an in vivo preparation, in which the intravenously administered drugs would reach the receptor sites through the blood stream with active pressure.

METHODS

Male guinea-pigs, weighing 350 to 450 g, were used under Nembutal® (sodium pentobarbital) anesthesia (35 mg/kg).

Detail of the recording technique of electrical and mechanical activity from the guinea-pig vas deferens in situ has been described in the previous report (7). In brief, the animal was laparotomized and one of the vasa deferentia was exposed. The opened abdominal cavity was filled with warm liquid paraffin. The hypogastric nerve was cut and the peripheral end was stimulated by square wave pulse (1 or 30 pulse/sec in frequency, 1 msec in duration and submaximal intensity). The electrical activity (junction potentials and spike potentials) of the vas deferens was led monopolarly through an extracellular electrode, which was made from a silver wire, 25 μ in diameter, and an AC-amplifier...
The mechanical response of the vas deferens was recorded using a pressure transducer and carrier amplifier (Nihon-Kohden RP-3).

For histochemical demonstration of catecholamines, the fluorescence method of Falck (11) was used. The small pieces of tissue were freeze-dried, exposed to formaldehyde gas at 80°C for one hour, embedded in paraffin, sectioned at 6 to 9 μ and mounted for fluorescence microscopy. A Zeiss fluorescence microscope with a Schott OG 4 filter was used. The source of activation light was an Osram HBO 200 high pressure mercury lamp with a Schott BG 12 filter.

Drugs used were adrenaline hydrochloride, bretylium tosylate, chlorpromazine hydrochloride, dibenamine hydrochloride, hexamethonium bromide, noradrenaline hydrochloride, tetraethylammonium chloride (TEA), tolazoline hydrochloride and yohimbine hydrochloride.

RESULTS

The electrical and mechanical responses to hypogastric nerve stimulation

The low frequent repetitive stimulation (up to 5 pulses/sec) of hypogastric nerve produced a small electrical fluctuation "junction potential" with marked facilitation following each impulse. The critical frequency of the stimulation for generating spike potentials was 2 to 3 pulses/sec.

The spike potentials were usually accompanied by contraction of the vas deferens. The degree of contraction in response to hypogastric nerve stimulation varied according to the stimulation frequency. The optimal stimulation frequency for the contractile response in situ was about 30 pulses/sec. After use of fairly high frequency stimulation

![Mechanical activity of whole vas deferens (upper trace) and electrical activity of three different parts of the vas deferens in response to hypogastric nerve stimulation (20 pulses/sec, 1 msec and submaximal intensity) in guinea-pig. I. Prostatic, II. Center, III. Epididymal. The recording electrodes were apart 0.5 cm from each other.](image)
(more than 20 pulses/sec), electrical after-discharge which was accompanied by sustained tonic contraction of the vas deferens, was frequently observed. The electrical responses of different parts of the vas deferens to hypogastric nerve stimulation were usually synchronous as shown in Fig. 1, in which three recording electrodes were separated 0.5 cm from each other.

Effect of noradrenaline

The intravenous injection of more than 5 μg/kg of noradrenaline produced a spike burst accompanied by tonic contraction, which usually continued for a few minutes. Many investigators have reported that the addition of noradrenaline into the bath augmented the contractile response of the vas deferens to hypogastric nerve stimulation (1, 12, 13). The intravenous injection of noradrenaline also augmented both the electrical and mecha-

![Fig. 2. Effect of noradrenaline on the junction potentials (A) and tension development and spike potential (B) in response to hypogastric nerve stimulation in guinea-pig vas deferens. Stimuli: (A) 1 pulse/sec, 1 msec and submaximal intensity (B) 20 pulses/sec, 1 msec and submaximal intensity Upper trace: control Lower trace: 10 minutes after the drug administration](image-url)
nical responses to hypogastric nerve stimulation 10 minutes after the drug administration, but the degree of the augmentation of the responses was not so dramatic compared with that of the in vitro preparation (Fig. 2). Perhaps there is no depletion of available transmitter substance in nerve terminals due to circulatory noradrenaline supply in the present in vivo experiment.

Effects of ganglionic blocking agents

The intravenous injection of ganglionic blocking agents such as hexamethonium (3 mg/kg) and TEA (20 mg/kg) had no effect on the spontaneous mechanical activity of the vas deferens. Both electrical and mechanical responses to hypogastric nerve stimulation were abolished by the intravenous injection of hexamethonium (3 mg/kg) and TEA (20 mg/kg), while the increased electrical and mechanical activities elicited by the intravenous injection of adrenaline (Fig. 3A, B) were not affected. The result is consistent with Sjöstrand's observation (14) that ganglionic blocking agents blocked the contractile response to hypogastric nerve stimulation in vitro, and later, the existence of adrenergic ganglionic cell bodies was confirmed histochemically (4). The present histochemical studies also confirmed the existence of adrenergic ganglion scattered near the prostata, seminal vesicle

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**FIG. 3A.** Effect of hexamethonium (3 mg/kg) on the mechanical and electrical response to hypogastric nerve stimulation (20 pulses/sec, 0.1 msec and submaximal intensity)

Upper trace: control
Middle trace: 10 minutes after the drug administration
Lower trace: 90 minutes after the drug administration
Note "after-discharge" in control tracing.
Fig. 3B. Effect of TEA (20 mg/kg) on the mechanical and electrical activities evoked by hypogastric nerve stimulation (2 pulses/sec and 20 pulses/sec, 1 msec and submaximal intensity) and intravenous injection of 5 µg/kg of adrenaline (lower trace).
Note that TEA blocked the nerve response, but not blocked the response to exogenous adrenaline.

Fig. 4. Histochemical demonstration of adrenergic ganglion existing near the prostate, seminal vesicle and vas deferens. Calibration 300 µ.
A: Non-fluorescent (presumably cholinergic), preganglionic fibers proceeding toward ganglionic cells.
B: Adrenergic ganglion.
C: Adrenergic fluorescent, postganglionic fiber emerging from ganglionic cell bodies. Also note the adrenergic nerve terminals innervating prostate and seminal vesicle (D).
and vas deferens. The ganglion contains two different sized adrenergic cells. The diameter of the larger type of cells was about 70 \( \mu \), while the smaller type was almost similar in size to the satellite cells. Adrenergic postganglionic fibers emerging from ganglionic cell bodies and presumably cholinergic preganglionic, non-fluorescent fibers proceeding toward the cells, were seen as shown in Fig. 4. Therefore it is most likely that ganglionic blocking agents act on the adrenergic cells, because the response to nerve stimulation was specifically depressed, while the response to exogeneously administered adrenaline was not blocked.

**Effect of bretylium**

The intravenous injection of bretylium (10 mg/kg), an adrenergic neuron blocking
agent, gradually depressed the electrical and mechanical response to hypogastric nerve stimulation. Junction potentials in response to low frequency nerve stimulation (1 pulse/sec) and spike bursts evoked by higher frequency stimulation (30 pulses/sec) were similarly reduced by bretylium. The evidence seems to be consistent with Morrison and Parkes' observation (15) that the contractile response of the vas deferens at different frequencies of stimulation was similarly depressed by bretylium.

**Effects of α-blocking agents**

The previous reports regarding the effects of various α-blocking agents upon the mechanical response of the vas deferens to hypogastric nerve stimulation in vitro are somewhat inconsistent. Ohlin and Strömblad (10) observed the enhancement of nerve responses by the administration of dihydroergotamine and phenoxybenzamine. However, Birmingham and Wilson (16) and Della et al. (17) have reported the depressive effects of the drugs on the transmurally stimulated preparation.

The intravenous injection of dibenamine (5 mg/kg) and chlorpromazine (3 mg/kg) caused a gradual depression of the electrical and mechanical responses to hypogastric nerve stimulation, while yohimbine (1 mg/kg) and tolazoline (5 mg/kg) potentiated the nerve response 30 minutes after drug administration. The present study has not dealt with the effects of unphysiologically high doses of α-blocking agents on the nerve response, although Burnstock and Holman (8) have described that high concentration (more than $5 \times 10^{-4}$ g/ml) of various adrenolytic drugs were needed to block the junction potentials in response to nerve stimulation and spontaneous discharge (miniature junction potential), suggesting resistance of the nerve response to various α-blocking agents, in vitro. The present study

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**Fig. 6.** Effects of yohimbine (1 mg/kg), tolazoline (5 mg/kg), chlorpromazine (3 mg/kg), dibenamine (5 mg/kg) and bretylium (10 mg/kg) on the contractile response to hypogastric nerve stimulation (20 pulses/sec, 1 msec and submaximal intensity).

Abscissa : time course (minute)

Ordinate : % changes of amplitude of contraction in response to hypogastric nerve stimulation after drug-administration.

- : increase - : decrease
allows the blocking agents to reach the \( \alpha \)-receptor site on the smooth muscle membrane through a physiological blood stream, but still a resistance of nerve response to \( \alpha \)-blocking agents was demonstrated.

**DISCUSSION**

It has been confirmed by many investigators that the muscular layers of the vas deferens are very richly and predominantly innervated by adrenergic fibers (4). The origin of junction potentials evoked by low frequency stimulation of the hypogastric nerve is considered to be noradrenaline released from the sympathetic nerve terminals, this conclusion being based on the following results. 1) The junction potentials were strongly depressed by pretreatment (5) or intravenous injection (6) of reserpine. 2) The junction potentials were also depressed by guanethidine and bretylium (8). 3) The junction potentials were not affected by atropine (8, 18). However, the junction potentials were not depressed by moderate doses of \( \alpha \)-blocking agents. Higher doses \( (5 \times 10^{-4} \text{g/ml}) \) of the drugs were needed to obtain the depressive effects on junction potentials in response to hypogastric nerve stimulation in the in vitro preparation (8). The present in vivo study also confirmed the insensitivity of junction potentials to \( \alpha \)-blocking agents, in which the drugs may act on receptor sites under more physiological condition through the blood capillaries than in vitro preparation.

In general, the response of urogenital organs to cholinergic nerve stimulation shows a resistance to atropine treatment (19, 20). It is most likely that resistance may be due to close contact (about 200 Å) between the nerve terminal and effector cell membrane, which was demonstrated by Richardson (21) and Merrillees et al. (22). It seems from their electronmicroscopic photographs that no barrier structure existed near the junctional region. This geometrical relationship between the nerve terminal and the receptor site might interfere with the action of adrenergic blocking drugs on the response to sympathetic nerve stimulation.

In regard to the effect of bretylium on the electrical activity of the vas deferens in vitro, Burnstock and Holman (8) have reported an increase in the spontaneous discharge, whereas the response to nerve stimulation was completely blocked 40 minutes after exposure to the drug. Unfortunately, the present extracellular recording method did not allow us to study the miniature spontaneous discharge, because it was difficult to distinguish the potential from noise level.

The synchronous appearance of the electrical response in different parts of the muscular layer of the vas deferens to nerve stimulation suggests that the nerve endings must be distributed widely among the smooth muscle cells. The present study reconfirmed pharmacologically and histochemically the existence of adrenergic ganglion associated with short adrenergic postganglionic fibers and non-adrenergic preganglionic fibers which had been previously demonstrated by Falck (4).
SUMMARY

The effects of ganglionic blocking agents and adrenergic neuron blocking agents upon the electrical and mechanical activities of the guinea-pig vas deferens in response to hypogastric nerve stimulation in vivo was described in the present report. In addition, histochemical observation of the adrenergic innervation in the tissue was also carried out. The results obtained are summarized as follows:

1. The junction potentials with marked facilitation were evoked by the repetitive stimulation (1 pulse/sec) of the hypogastric nerve of the guinea-pig vas deferens, in vivo. Increasing the stimulation frequency up to 3 to 4 pulses/sec usually generated spike potentials accompanied by tension development of the tissue.

2. The intravenous injection of 5 µg/kg of noradrenaline produced a spike burst associated with tonic contraction of the vas deferens. Furthermore, noradrenaline (5 µg/kg) slightly potentiated both the electrical and mechanical responses to hypogastric nerve stimulation 10 minutes after drug administration.

3. The intravenous injection of ganglionic blocking agents, such as hexamethonium (3 mg/kg) or TEA (20 mg/kg), blocked both electrical and mechanical responses of vas deferens to hypogastric nerve stimulation, while the response to exogenous adrenaline was not affected. A histochemical study demonstrated the existence of adrenergic ganglion near the prostata, seminal vesicle and vas deferens.

4. The amplitude of junction potentials, the number of spike potentials and the amplitude of the contraction elicited by hypogastric nerve stimulation were gradually depressed and sometimes abolished 30 minutes after the intravenous administration of bretylium (10 mg/kg).

5. The intravenous injection of chlorpromazine (3 mg/kg) and dibenamine (5 mg/kg) produced gradual depression on the electrical and mechanical responses to hypogastric nerve stimulation. However, neither tolazoline (5 mg/kg) nor yohimbine (1 mg/kg) depressed both responses but rather potentiated them.

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REFERENCES

1) HUKOVIC, S.: Br. J. Pharmac. Chemother. 16, 188 (1961)
2) BENTLEY, G.A.: Br. J. Pharmac. Chemother. 19, 85 (1962)
3) BENTLEY, G.A. AND SABINE, J.R.: Br. J. Pharmac. Chemother. 21, 190 (1963)
4) FALCK, B., OWMAN, C.H. AND SJOSTRAND, N.O.: Experientia 21, 98 (1965)
5) BURNSTOCK, G. AND HOLMAN, M.E.: J. Physiol. 160, 461 (1962)
6) NAKANISHI, H. AND TAKEDA, H.: Jap. J. Pharmac. 15, 199 (1965)
7) TAKEDA, H. AND NAKANISHI, H.: Ann. Rept. Shionogi Res. Lab. 15, 163 (1965)
8) BURNSTOCK, G. AND HOLMAN, M.E.: Br. J. Pharmac. Chemother. 23, 600 (1964)
9) BOYD, H., CHANG, V. AND RAND, M.J.: Br. J. Pharmac. Chemother. 15, 525 (1960)
10) OHLIN, P. AND STRÖMBLAD, B.C.R.: Br. J. Pharmac. Chemother. 20, 299 (1963)
11) Falck, B.: *Acta physiol. scand.* 56, suppl. 197 (1962)
12) Sjöstrand, N.O.: *Nature, Lond.* 192, 1190 (1961)
13) Yamamoto, H. and Nakanishi, H.: *Jap. J. Pharmac.* 14, 256 (1964)
14) Sjöstrand, N.O.: *Acta physiol. scand.* 54, 306 (1962)
15) Morrison, A.B. and Parkes, M.W.: *J. Pharm. Pharmac.* 16, 647 (1964)
16) Birmingham, A.T. and Wilson, A.B.: *Br. J. Pharmac. Chemother.* 21, 569 (1963)
17) Della Bella, D., Benelli, G. and Gandini, A.: *J. Pharm. Pharmac.* 16, 779 (1964)
18) Takeda, H. and Nakanishi, H.: *Jap. J. Smooth Muscle Res.* 1, 42 (1965)
19) Ursillo, R.C. and Clark, B.B.: *J. Pharmac. exp. Ther.* 118, 338 (1956)
20) Ambache, N.: *Pharmac. Rev.* 7, 467 (1955)
21) Richardson, K.C.: *J. Anat.* 96, 427 (1962)
22) Merrillees, N.C.R., Burnstock, G. and Holman, M.E.: *J. cell. Biol.* 19, 529 (1963)