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Pharmacological Studies on Human Vas Deferens

—Coexistence of Adrenergic and Cholinergic Receptors, and Effect of Diazepam—

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ABSTRACT

This study aimed to investigate the autonomic innervations of human vas deferens and the effect of diazepam, a benzodiazepine sedative antianxiety drug, on the smooth muscle contractility of vas deferens. The specimens were obtained from healthy volunteers undergoing elective vasectomy with local anesthesia.

The muscle preparation did not show any spontaneous contraction, but showed a good contraction induced by norepinephrine exerting the strongest response at 33℃. Phentolamine inhibited the norepinephrine-induced contraction concentration-dependently. Isoproterenol, a beta-adrenergic agonist evoked a considerable extent of contraction, and this contractile activity was antagonized by propranolol, a beta-adrenergic blocking agent. Acetylcholine induced a dashing contraction of the human vas deferens, and atropine, a muscarinic receptor blocking agent abolished the acetylcholine-induced contraction. Diazepam inhibited the norepinephrine-induced contraction in a concentration dependent manner.

These results suggest that the smooth muscle of human vas deferens has cholinergic muscarinic and beta adrenergic receptors as well as the predominant alpha adrenergic receptor. Diazepam inhibits the motility, especially norepinephrine-induced contraction of human vas deferens.

Key Words: Human, Vas deferens, Adrenergic, Cholinergic, Diazepam

INTRODUCTION

The motility of the human vas deferens is an important factor in the reproductive function.

There is a little controversy about the motor innervation in human vas deferens. Most of the investigators who studied the motility of human vas deferens suggested that the motor innervation is mainly adrenergic in nature (Martins *et al.*, 1940; Birmingham, 1968; Ventura *et al.*, 1973; McLeod *et al.*, 1973; Hepperlen, 1976; Anton and McGrath, 1977; Ratnasooriya *et al.*, 1979; Kawamoto *et al.*, 1986). Ventura *et al.* (1973) reported that it was confirmed that the motility of the vas deferens *in vivo* was under the control of sympathetic fibers which release norepinephrine. Martins, *et al.* (1940) reported that human vas deferens contracted by acetylcholine, nicotine, histamine, and barium ion as well as ephedrine and epinephrine, suggesting the motor innervation involved not only adrenergic but also cholinergic fibers.

Recently, Alm (1982) also reported that acetylcholinesterase positive nerve fiber was found in the connective tissue of the mucosa. We were interested in the autonomic innervation in human vas deferens and tried to find the responsiveness of the vas deferens to various autonomic drugs through an experiment with isolated muscle preparation.

Benzodiazepines, a sedative drug commonly used to treat anxiety, was reported to inhibit intestinal motility in guinea pig ileum (Hullihan *et al.* 1983). Rampe and Triggle (1987) observed the interaction between benzodiazepines and voltage

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dependent calcium channel. These findings lead us to suppose that a long term administration with benzodiazepines might suppress the male reproductiveness with inhibition of the transport of spermatozoa.

In this study, we have two objectives, to investigate what type of autonomic receptor responds to any autonomic agonist resulting in contraction, and if diazepam inhibits the contractility of smooth muscle in human vas deferens.

MATERIALS AND METHOD

Preparation of isolated vas deferens

Two segments of the vas deferens from both sides were obtained from healthy individuals undergoing elective vasectomy. The volunteers were between 32 to 45 years old, and the surgery was performed under local anesthesia (0.5% lidocaine, 5 ml, subcutaneous infiltration). The segments were transported to the laboratory in the ice cold balanced salt solution described by McLeod (McLeod *et al.*, 1973).

One end of the isolated segment of 3 to 5 mm long was tied with silk thread and was anchored at the bottom of the muscle bath, and the silk thread tying the other end was hooked at an isometric tension transducer (Narcobiosystem, myograph F -60). The tensions of the muscle preparations were recorded on a physiograph (Narcobiosystem, MK-IV-P).

The isolated organ bath in which the muscle preparation was mounted was containing 15 ml of oxygenated balanced salt solution. The bathing solution devoid of glucose were composed as following (millimoles per litter): NaCl, 130.0; KCl, 11.3; $CaCl_2$, 0.2; $MgSO_4$, 0.1; $NaHCO_3$, 11.9; pH= 7.4.

The muscles in the baths were stretched passively with 2.0 grams of initial tension and were given at least 30 minutes of time lapse to equilibrate. To search the optimal temperature for the muscle to respond the most effectively to norepinephrine, the muscles were equilibrated in various temperature (23 to 36℃) to be added with norepinephrine increasing in concentration cumulatively.

Responses to drugs and data analysis

These experiments were executed at the temperature of 33℃. When the muscle equilibrated, cumulative concentration-responses to adrenergic and cholinergic agonists were observed before and after exposures to single concentrations of antagonists.

The drugs used for this study were as following: noradrenaline bitartarate (Sigma; norepinephrine), phentolamine mesylate (Ciba-Geigy, donated), isoproterenol (Sigma), propranolol (Sigma), acetylcholine chloride (Tokyo Kasei), atropine sulfate (Kook-Jeon).

The concentration-response to norepinephrine was checked in the presence of various concentrations of diazepam.

Data were revealed as mean±S.E. and evaluated by Student's T-test.

RESULTS

Optimal temperature for the contraction of human vas deferens

Under the condition of this experiment, the muscle pieces of human vas deferens did not show any spontaneous contractile activity, but they contracted responding to norepinephrine. The muscle preparations at relatively lower concentration (0.01 mM) of norepinephrine showed simple slight elevation of tone, but at higher concentrations (0.1 mM and 1 mM), they showed repeated phasic contractions (Fig. 1). As shown on Fig. 3, the amplitude of phasic contraction increased very sharply from the concentrations of 0.01 mM to 0.03 mM, and tended to decrease at further higher concentrations. The frequency of phasic contraction increased in a concentration dependent manner responding maximally at 0.1 mM.

The strongest responses in amplitude and frequency of the phasic contractions to norepinephrine at every concentration employed in this expriment were attained at the temperature of 33℃ (Fig. 2). The most effective concentration of norepinephrine at this temperature (33℃) was 0.1 mM.

Effects of autonomic drugs on the contractility of human vas deferens

Patterns of contraction induced by norepinephrine, isoproterenol and acetylcholine were tonic or phasic. Noticeable repeated phasic contractions were observed, when the muscle preparations were exposed to 0.1 mM of norepinephrine, 10 mM of

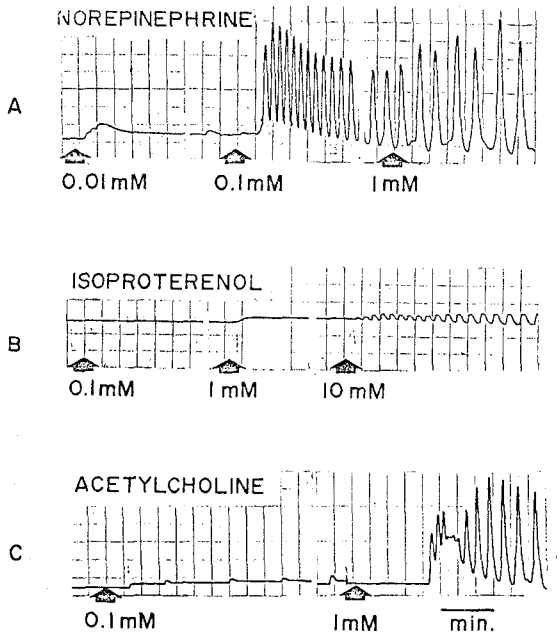

Fig. 1. Representative exhibition of the contractile responses of human vas deferens to norepinephrine (A), isoproterenol (B) and acetylcholine (C)

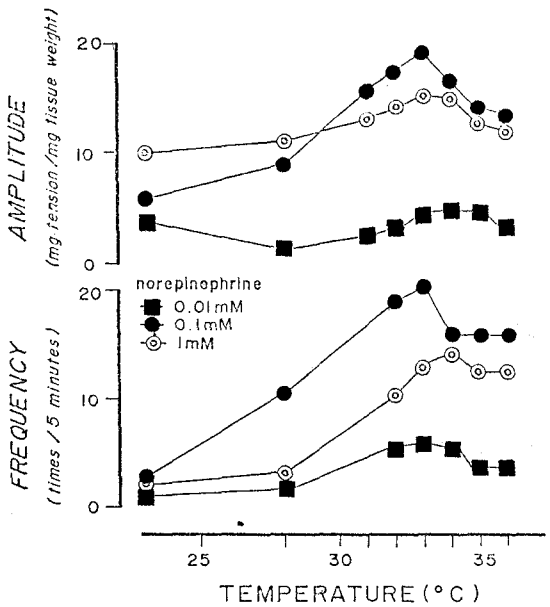

Fig. 2. Effect of temperature on the contractility of human vas deferens responding to norepinephrine in various concentrations

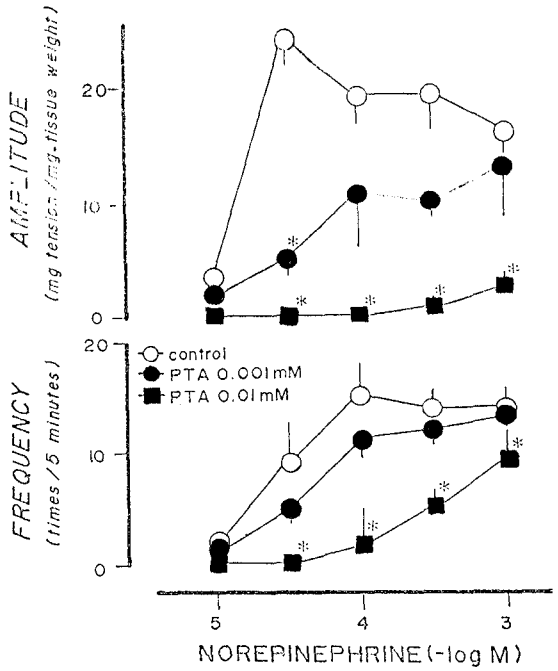

Fig. 3. Concentration-response of human vas deferens to norepinephrine in the presence of phentolamine
PTA; Phentolamine
Data are revealed as Mean±S.E.
* Significant, $p<0.05$ by Student's t-test

isoproterenol, or 1 mM of acetylcholine (Fig. 1).

The cumulative-concentration response curves of norepinephrine were dipicted in Fig. 3. In control, norepinephrine evoked the maximal response in amplitude (25.5±0.60 mg tension/mg tissue weight) at 0.03 mM, and in frequency (16.3±1.80 times/5 minutes) at 0.1 mM. In the groups pretreated with phentolamine (0.001 mM and 0.01 mM), the amplitude and frequency of the contractions were reduced concentration-dependently. The decrease in amplitude was significant ($p<0.05$) at 0.001 mM of phentolamine, and the decrease in frequency was at 0.01 mM.

Isoproterenol exerted a weak contraction at a concentration of 1 mM with 10.38 (mg tension/mg tissue weight) of amplitude, 6.18 (times/5 minutes) of frequency. Propranolol (0.01 mM) abolished the isoproterenol-induced contraction (Fig. 4).

Fig. 5 shows the cumulative concentration-response curves of acetylcholine. Acetylcholine, 1 mM, evoked the maximal response (amplitude; 14.88 mg tension/mg tissue weight, frequency; 1.76

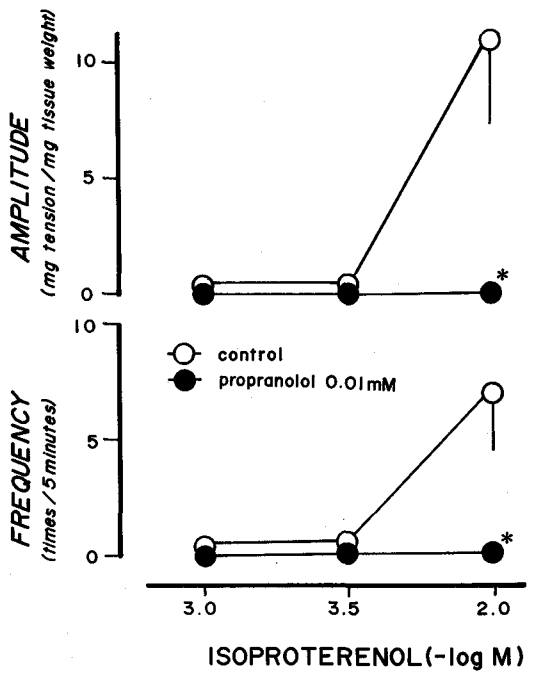

Fig. 4. Contractile response of human vas deferens to isoproterenol
In the presensce of propranolol, isoproterenol-induced contraction was completely abolished for the response curve to be flat.
Data are revealed as Mean±S.E.
* Significant, $p < 0.05$ by Student's t-test

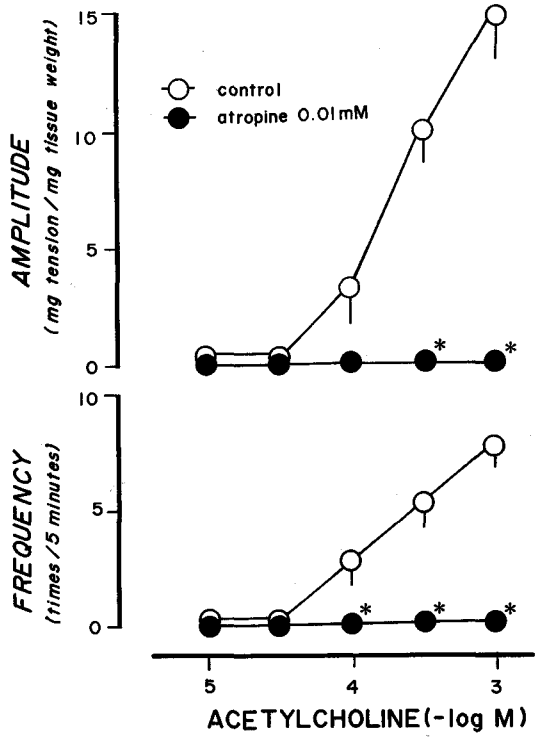

Fig. 5. Concentration-response of human vas deferens to acetylcholine
In the presence of atropine, acetylcholine-induced contraction was completely abolished for the response curve to be flat.
Data are revealed as Mean±S.E.
* Significant, $p < 0.05$ by Student's t-test

times/minute). The contraction in response to acetylcholine was abolished by the pretreatment with atropine (0.01 mM).

Effects of diazepam on the norepinephrine-induced contraction in the vas deferens

Diazepam did not affect the basal tone, but reduced the norepinephrine-induced contraction in a concentration-dependent manner. As shown in figure 6, the increase in frequency induced by norepinephrine tended to be inhibited by incubation in diazepam, but it was not significant. The amplitude of norepinephrine-induced contraction was reduced in the presence of diazepam concentration-dependently (significant, $p < 0.05$).

DISCUSSION

Spermatozoa can live for many weeks in the male genital ducts, once they are ejaculated in the semen their maximal life span is only 24 to 48 hours at body temperature, and it has often been stated that the temperature of scrotum is maintained below body temperature for the efficient spermatogenesis (Guyton, 1986). In this experiment, the muscle preparations obtained by vasectomy was originated from the scrotal portion, and showed the strongest response to norepinephrine at 33℃. It looks justifiable that the extraabdominal portion of the vas deferens exerts the most efficient motility at a lower temperature than that of the body itself.

Spontaneous motility of the isolated human vas deferens has been described by Ventura *et al.* (1973) and McLeod *et al.* (1973). Ventura *et al.* (1973) reported that the isolated human vas deferens from cadavers and from patients under general

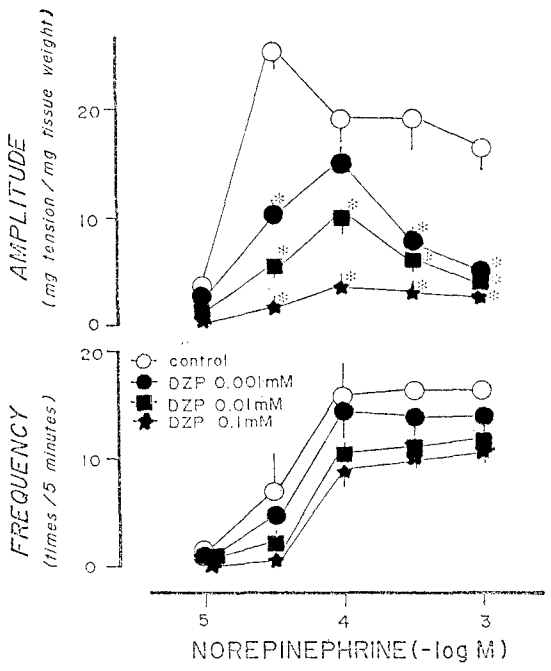

Fig. 6. Concentration-response of human vas deferens to norepinephrine in the presence of diazepam
DZP; Diazepam
Data are revealed as Mean±S.E.
* Significant, $p<0.05$ by Student's t-test

anesthesia displayed spontaneous motility, but the vasa from vasectomies under local or spinal anesthesia showed no spontaneous activity. They guessed that the spinal center of sympathetic innervation controlling the motility of the vas deferens was affected by local anesthetics injected. McLeod *et al.* (1973) explained that in Ventura's experiment, the diffusion of local anesthetics during surgery might have suppressed the spontaneous contractility. Ratnasooriya *et al.* (1979) investigated the motility of human vas deferens with 44 specimens from patients vasectomized under local anesthesia, and reported that 45% of them showed spontaneous contraction.

Hepperlen *et al.* (1976) noted no spontaneous activity in any preparation of the vas deferens from volunteers under local or spinal anesthesia. All of these investigators, whoever noted the spontaneous activity or not, observed fairly good responses of their preparations to norepinephrine. These findings declare the presence of receptors interacting with norepinephrine, supposedly alpha- or beta-adrenergic in nature, on the smooth muscle in human vas deferens. In our study, the surgery was performed as quickly as possible using the least concentration of lidocaine to prevent the diffusion of the local anesthetic drug into the muscle layer of the vas deferens.

But we could not recognize any spontaneous contraction using a recording system which is sensitive enough to detect the force (200 mg) of spontaneous contraction observed by Ventura *et al.* (1973). The muscle preparation in the bath was just quiescent in the equilibration period, but later it displayed an excellent contractile response to norepinephrine.

Phentolamine an alpha-adrenergic blocking agent attenuated the conractile response to norepinephrine concentration-dependently, and there is no doubt that human vas deferens has the alpha-adrenergic receptor.

Isoproterenol, a predominantly beta-adrenergic agonist, stimulates the beta 2-adrenergic receptor in smooth muscle. McLeod *et al.* (1973) reported that isoproterenol failed to produce either excitatory or inhibitory response. This report is different from our result which showed a contractile response of the muscle to isoproterenol. As exhibited on figure 1, isoproterenol elevated the basal tone of the preparation that had been quiescent previously, and exerted phasic contractions to a considerable extent. Moreover, such an elevation of basal tone or phasic contraction was completely abolished by incubation in propranolol.

Unsatisfactorily, McLeod *et al.* (1973) did not describe the concentration of isoproterenol, which is presumed to be lower than the concentration we employed. As a matter of fact, 10 mM is a high concentration for a drug, but such a concentration may not be too high for the receptors interacting with the neurotransmitters at neuroeffector junctions. However, our preparations contracted by isoproterenol, and the isoproterenol-induced contraction was antagonized by propranolol, a beta-adrenergic blocking agent.

There is another controversy about the contribution of cholinergic receptors to the contractility of the vas deferens. Ventura *et al.* (1973) reported that the human vas deferens did not show any contractile response to acetylcholine, and McLeod *et al.* (1973) reported that they were not able to show any consistent response to acetylcholine even in the presence of acetylcholinesterase inhibitor, and the tissues failed to respond to nicotine also. These reports suggested that there was no distribution of cholinergic innervation including auto-

nomic ganglion, justly parasympathetic, in human vas deferens.

On the other hand, there are some reports suggesting the exsistence of cholinergic innervation in human vas deferens. Martins *et al*. (1940) reported that the vas deferens and epididymis responded to acetylcholine, and the responses were less pronounced and less consistent than that to epinephrine. Recently as a result of a histochemical study on human vas deferens, some nerve terminals displaying acetylcholinesterase activity and occuring even more frequently than the adrenergic nerve terminals were demonstratd by Alm (1982). In our study, the muscle preparations responded to acetylcholine with a consistent dashing contraction mostly phasic and partly tonic. Atropine, a muscarinic receptor blocking agent, totally antagonized this acetylcholine-induced contraction and let the muscle sit quiet.

To examine the effect of diazepam to the motility of vas deferens, we chose norepinephrine as an indicator. Diazepam did not change the basal tone of muscle preparation under the condition given in this study, and norepinephrine was the most sensitive agonist for the human vas deferens to contract. In guinea pig ileum, diazepam inhibited the contraction induced by carbachol, histamine, potassium chloride and calcium ion; and the involvement of calcium ion in the mechanism of inhibitory action of diazepam was suggested by the author (Hullihan *et al*., 1983).

In our study, diazepam depressed the concentration-response curve of norepinephrine both the amplitude and frequency. A precise evaluation of the data could not be done, for the numbers of specimens were not many enough because of the difficulty in collecting the volunteers. However, apparently, the higher concentration of diazepam depressed the slope of the concentration-response curve the flatter especially in amplitude of phasic contraction. It is assumed that a long term administration of diazepam to a male patient may influence the reproductive function somewhat disadvantageously. Further investigations to elucidate the mechanism of the inhibitory action of diazepam on the motility of human vas deferens is required to get more informations helpful to dissolve the above mentioned assumption.

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=국문초록=

인체 정관의 약리학적 검색
—아드레날린성 및 콜린성 수용체의 공존과 Diazepam의 작용—

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인체 정관 평활근에서 각종 자율신경전달체 수용체의 유무를 조사하고 benzodiazepine계의 진정-항불안제인 diazepam이 평활근 운동성에 미치는 작용을 관찰하기 위하여, 32내지 45세의 건강한 지원자로부터 정관절편을 얻었다. 정관 절제술은 국소마취하에 시행되었고, 정관절편의 수축력 측정은 등장성장력측정기에 의하였다. 적출장기실험조 내에서 정관절편의 자율수축은 관찰되지 않았으나, norepinephrine에 대한 반응성은 33℃에서 가장 예민하였던 바, 이 norepinephrine에 의한 농도의존적 수축력증가작용은 알파-아드레날린성 차단제인 phentolamine에 의해 억제되었다. 또 인체 정관절편은 본 실험의 조건하에서 isoproterenol에 의하여 수축하였고, 이 수축작용은 베타-아드레날린성 차단제인 propranolol에 의하여 완전히 제거되었다. 동시에 인체 정관절편은 acetylcholine에의해서도 비교적 강하게 수축하였고, 이 수축작용은 콜린성 무스카린성 차단제인 atropine에 의하여 완전히 억제되었다. Diazepam은 norepinephrine에 의한 수축을 농도 의존적으로 억제하였다.

이상의 결과를 종합하면, 인체 정관 평활근은 체온보다 낮은 33℃에서 그 활동성이 가장 강하고, 자율신경에 대하여서는 아드레날린성 및 콜린성 수용체가 공존하고 있으며, diazepam은 그 수축력을 약화시킨다고 사료된다.