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Effects of St-587 on the α-adrenoceptors in the Bisected Rat Vas Deferens

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Abstract—The effects of the α-adrenoceptor agonist St-587 have been studied on the twitch responses induced by field stimulation in the prostatic portion of rat vas deferens. Moreover the drug's influence on the unstimulated prostatic and epididymal halves of rat vas deferens has also been determined. Alone and after addition of yohimbine (0·3 μM) it enhanced in a concentration-dependent manner the twitch responses in the prostatic half. Prazosin competitively antagonized ($pA_2 = 8{\cdot}41 \pm 0{\cdot}03$) this effect. The enhancing effect of St-587 was not reduced in reserpinized animals. These results suggest that post-synaptic α_1-adrenoceptors are involved in the potentiation of twitch responses induced by St-587. When α_1-adrenoceptors were blocked by prazosin (0·1 μM), St-587 partially inhibited the twitch responses of the prostatic portion of rat vas deferens ($E_{max} = 49{\cdot}5 \pm 3{\cdot}5\%$). Yohimbine completely reversed the inhibitory effects of both St-587 and clonidine. Furthermore St-587 antagonized the inhibitory effects of clonidine on twitch responses. Thus it appears that St-587 also behaves as a partial agonist of presynaptic α_2-adrenoceptors in this portion of rat vas deferens, but it did not induce contractions in the unstimulated prostatic half of the vas deferens. However, it competitively antagonized the α_1-adrenoceptor agonist phenylephrine by acting as an antagonist of prostatic postsynaptic α_1 adrenoceptors. These α_1-adrenoceptors are probably different from those that mediate the twitch enhancing response to St-587 in that portion. On the other hand, St-587 was a partial agonist of α_1-adrenoceptors in the epididymal half. These results suggest that a different efficiency of stimulus-response mechanism is operating after postsynaptic α_1-adrenoceptor activation in each one of the unstimulated portions of rat vas deferens.

There is now an important body of evidence to substantiate that the prostatic and epididymal portions of the rat vas deferens differ in both their motor transmission (McGrath 1978; Brown et al 1983) and their postsynaptic sensitivity to different α-adrenoceptor agonists (Kasuya & Suzuki 1979; Moore & Griffiths 1982; Vardolov & Pennefather 1976). There is also evidence that α_1-agonists show two different postjunctional responses in the vas deferens: potentiation of nerve evoked contractions and spontaneous contractions of smooth muscle (MacDonald & McGrath 1980). In addition, α_2-adrenoceptor agonists induce an inhibition of the twitch contraction of the nerve-stimulated rat vas deferens through the activation of presynaptic α_2-adrenoceptors (Drew 1977; Doxey et al 1977; MacDonald & McGrath 1980). St-587 (2-(2-chloro-5-trifluoromethylphenylimino)imidazolidine nitrate) is a selective α_1-adrenoceptor agonist that has been used as a tool for the classification of α-adrenoceptors (De Jonge et al 1981; Kobinger & Pichler 1982). However, it has been shown to have some α_2-adrenoceptor antagonist properties in the pithed rat (Pichler & Kobinger 1985). The present study was designed to evaluate the action of St-587 on the different α-adrenoceptors in the bisected rat vas deferens, in an attempt to gain more insight into the functional characteristics of both portions.

Materials and Methods

General

Male Sprague-Dawley rats (300-325 g) were killed by cervical dislocation and exsanguination. Both vasa deferentia were removed, cleaned and bisected into prostatic and epididymal portions. The corresponding half was set up in isolated organ baths containing 20 mL of Krebs bicarbonate solution of the following composition (in mM): NaCl 112·0; KCl 4·7; $CaCl_2$ 2·5; KH_2PO_4 1·1; $MgSO_4$ 1·2; $NaHCO_3$ 25·0 and glucose 11·1. The solution was maintained at 32 ± 0·5 °C and gassed with 95% O_2 and 5% CO_2. The organ responses against 0·5 g tension were recorded by means of an isometric transducer (UF-1) on an OmniScribe pen-recorder. At least 45 min was allowed to elapse before starting the experiment. Propranolol (1 μM), desmethylimipramine (0·1 μM), and normetanephrine (1 μM) were present continuously in the medium throughout the experiment to block β-adrenoceptors, neuronal and extraneuronal uptake, respectively. Where indicated prazosin (0·1 μM) or yohimbine (0·3 μM) were also present in Krebs solution to block α_1- or α_2-adrenoceptors, respectively.

Studies in the prostatic half

Platinum ring electrodes were placed above and below the prostatic half of the vas deferens and continuous field stimulation was carried out by square wave pulses of 3 ms duration and supramaximal voltage (20-30 V) at a frequency of 0·1 Hz (Ealing Stimulator). When the twitch responses to field stimulation became stable, the agonist was added to the bath in a cumulative concentration schedule every 3–5 min until the concentration-response curve was obtained. In a first set of experiments, the inhibition of twitch responses induced by St-587 in the presence of 0·1 μM of prazosin, or clonidine, was studied. The reversal of the inhibitory effects of the agonists by antagonists was also determined.

In a second group of experiments the enhancing effect of the twitch responses induced by St-587 was studied in the presence of yohimbine (0·3 μM). Control or tissues previously incubated with prazosin (10, 30 and 100 nM) were used and

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concentration-response curves obtained. The antagonist potency of prazosin was evaluated as pA_2 calculated from Schild plots (Arunklakshana & Schild 1959). In some experiments, rats were pretreated with reserpine (5 mg kg^{-1} i.p.) 24 h before to assess the effects of St-587 as described. Vasa deferentia from control (saline) or reserpine treated rats were also taken for biochemical determination of noradrenaline content by spectrofluorimetric assay (Laverty & Taylor 1968).

In a final group of experiments non-stimulated prostatic portions of vas deferens were used and concentration-response curves of isometric contractions with phenylephrine controls or in the presence of St-587 (1–10 μM) were constructed for each preparation. In this way it was possible to calculate the pA_2 values and the slopes of Schild plots (Arunklakshana & Schild 1959) in each experiment for the antagonism of St-587 versus phenylephrine.

Studies in the epididymal half

Cumulative concentration-response curves of isometric contractions were obtained for phenylephrine and St-587 in each tissue and the pD_2 (negative $\log_{10}$ of molar concentration of agonist producing a response 50% of its maximal effect) and maximal contractile response relative to noradrenaline (100 μM) were calculated. The concentration-response curves either control and in the presence of prazosin (10, 30 and 100 nM added 5 min before starting each curve) were also constructed for each preparation. In this situation, it was possible to calculate the pA_2 values and the slopes of the Schild plots (Arunklakshana & Schild 1959) in each experiment.

Statistics

All the results are expressed as the mean ± s.e. of the mean. Statistical significance was evaluated using a Student's *t*-test. The differences were significant when $P < 0.05$.

Drugs

The drugs used were: clonidine HCl (Boehringer Ingelheim), prazosin HCl (Pfizer), (±)-propranolol (Fides), desmethylimipramine HCl (Ciba-Geigy), normetanephrine HCl

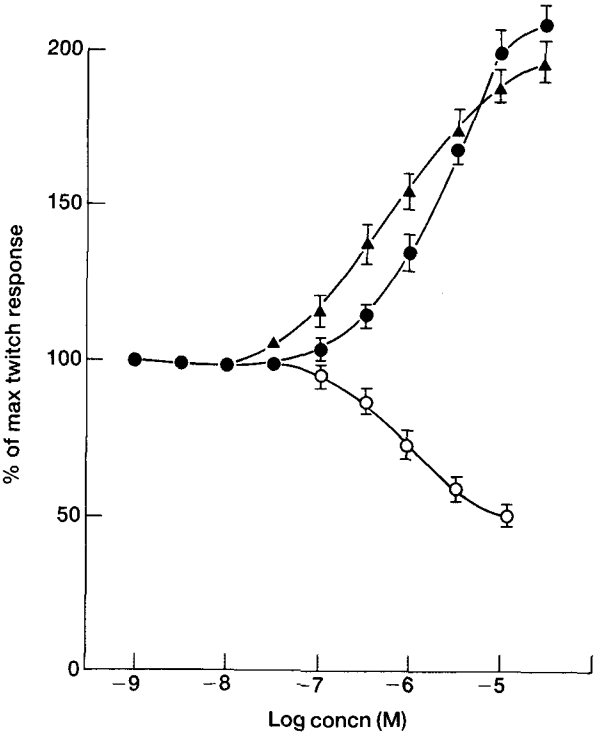

FIG. 1. Concentration-response curves of the effects of St-587 on twitch contractions of the prostatic half of rat vas deferens. (●———●) control, (▲———▲) in the presence of 0·3 μM of yohimbine and (○———○) in the presence of 0·1 μM of prazosin. Abscissa: logarithm of drug molar concentration. Ordinate: % of maximal twitch contraction. Each point is the mean of at least six experiments. Vertical bars show standard errors of the mean.

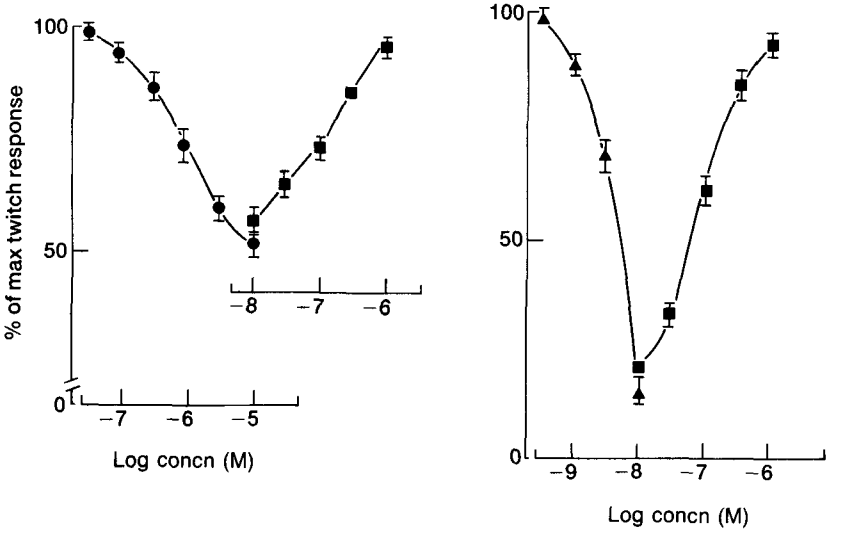

FIG. 2. Concentration-response curves of the inhibitory effects of St-587 (●———●) in the presence of 0·1 μM prazosin, and clonidine (▲———▲) on twitch contractions of the prostatic half of rat vas deferens and their antagonism by yohimbine (■———■). The concentration-response curves with yohimbine were obtained with increasing addition of antagonist after the maximal inhibitory effect with agonist was achieved. Abscissa: logarithm of drug molar concentration. Ordinate: % of maximal twitch contraction. Each point is the mean of at least six experiments. Vertical bars show standard errors of the mean.

(Calbiochem), St-587 (Boehringer Ingelheim), yohimbine HCl (Sigma), (−) noradrenaline bitartrate (Sigma), and reserpine (Serpasol, Ciba-Geigy).

Results

Effects of St-587 and clonidine on electrically evoked twitch responses

In prostatic portions of rat vas deferens St-587 (0·1–10 μM) potentiated, in a concentration-dependent fashion, the twitch contraction obtained with continuous field stimulation without increasing the basal tension. The addition of 0·3 μM of yohimbine increased this enhancing effect (Fig. 1). In contrast, when 0·1 μM prazosin was included in the Krebs solution, St-587 inhibited the twitch response in a concentration-dependent manner and the maximal inhibitory effect (49·5 ± 3·5%, n = 6) was obtained with 10 μM of the drug (Fig. 1). Clonidine also inhibited the twitch responses in a concentration-dependent manner. The inhibition of twitch responses induced by both St-587 and clonidine could be restored to control values by the cumulative addition of yohimbine (0·01–1 μM) to the bath (Fig. 2).

Prazosin (10–100 nM), in the presence of 0·3 μM yohimbine, shifted to the right the concentration-response curve of the enhancing effect of the twitch elicited by St-587 (Fig. 3). The antagonism of prazosin was competitive with a pA_2 of 8·41 ± 0·03 and a slope of Schild plot of 1·01 ± 0·02 (n = 6). In rats pretreated with reserpine (5 mg kg^{-1} i.p. 24 h) the noradrenaline content of vas deferens was reduced to less than 1% of control values and the enhancing effect also appeared.

The presynaptic inhibitory effect of clonidine with the inclusion of 0·1 μM prazosin in the Krebs solution was similar to that obtained without it. This inhibitory effect, also in the presence of prazosin, was clearly reversed by the graded addition of St-587 (Fig. 4). When the animals were previously treated with reserpine (5 mg kg^{-1} i.p. 24 h) St-587 restored the presynaptic inhibitory effect of clonidine in the same way.

Effects of St-587 on unstimulated rat vas deferens

St-587 (0·1–10 μM) did not modify the basal tension of the prostatic half. Higher concentrations (30–300 μM) induced only rhythmic contractions without increasing the basal tension. On the other hand, the concentration-response curves of phenylephrine were shifted to the right by increasing concentrations of the drug (1–10 μM) (Fig. 5). This antagonism was competitive with a pA_2 for St-587 against phenylephrine of 5·82 ± 0·02 and a slope of Schild plot of 1·02 ± 0·01 (n = 6).

Phenylephrine (0·1–100 μM) and St-587 (0·1–100 μM) elicited concentration-dependent contractions in the epididymal half of rat vas deferens. The pD_2 values and maximal effect are shown in Table 1 and, as can be seen, St-587 behaved as a partial agonist. This occurred not only under conditions of inhibition of neuronal and extraneuronal uptake with β-adrenoceptors but also without them (results not shown). The concentration-response curves of both agonists were antagonized by increasing concentrations of

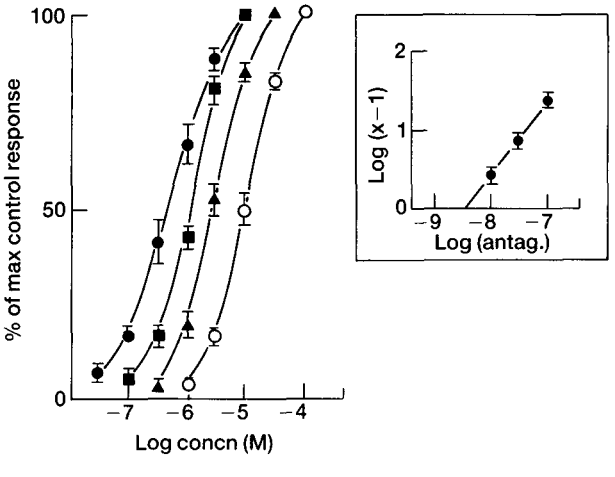

FIG. 3. Concentration-response curves of the enhancing effect of St-587, in the presence of 0·3 μM yohimbine, on the twitch contractions of the prostatic half of rat vas deferens and its antagonism by prazosin. (●———●) control; (■———■) in the presence of 0·01 μM prazosin; (▲———▲) in the presence of 0·03 μM prazosin and (○———○) in the presence of 0·1 μM of prazosin. Abscissa: logarithm of drug molar concentration. Ordinate: % of maximal control response. Inset shows the Schild plot for prazosin versus alinidine. X is the concentration ratio. Vertical bars show standard errors of the mean. Each point is at least the mean of six experiments.

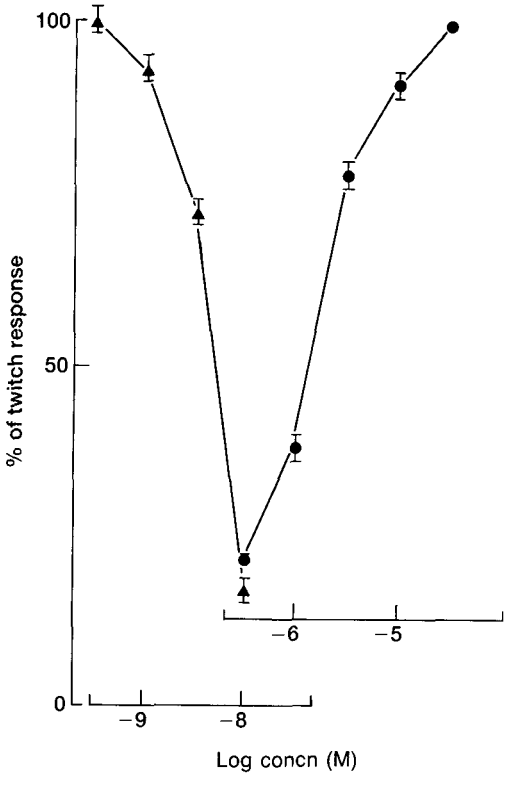

FIG. 4. Concentration-response curves of the inhibitory effects of clonidine in the presence of 0·1 μM prazosin (▲———▲) on twitch contractions of the prostatic half of rat vas deferens, and its antagonism by St-587 (●———●). The concentration-response curve with St-587 was obtained with increasing addition of antagonist after the maximal inhibitory effect with agonist was achieved. Abscissa: logarithm of drug molar concentration. Ordinate: % of maximal twitch contraction. Each point is the mean of at least six experiments. Vertical bars show standard errors of the mean.

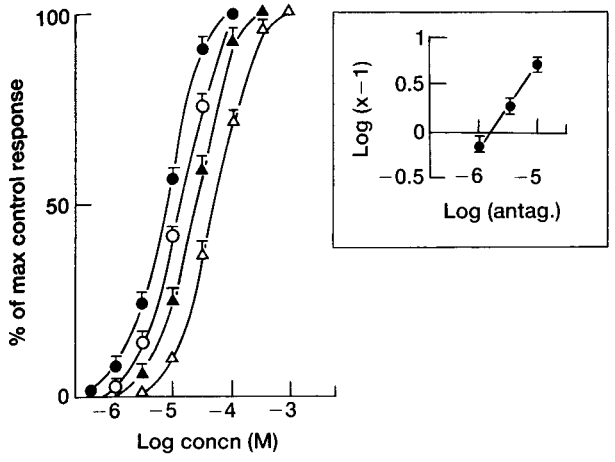

FIG. 5. Concentration-response curves of the contractile effects of phenylephrine in the unstimulated prostatic half of rat vas deferens and its antagonism by St-587 (●———●) control; (○———○) in the presence of 1 μM St-587; (▲———▲) in the presence of 3 μM St-587; (△———△) in the presence of 10 μM St-587. Abscissa: logarithm of drug molar concentration. Ordinate: % of maximal control response. Inset shows the Schild plot for St-587 against phenylephrine. X is the concentration ratio. Vertical bars show standard errors of the mean. Each point is at least the mean of six experiments.

prazosin (10–100 nM) (results not shown). From Table 2 it may be seen that the slopes of Schild plots were not significantly different from the theoretical value of 1 and the pA_2 values of prazosin against the two agonists were identical.

Discussion

St-587 has been described as a selective α_1-adrenoceptor agonist (De Jonge et al 1981; Kobinger & Pichler 1982). Recently, it has been shown to have some α_2-adrenoceptor antagonist properties (Pichler & Kobinger 1985). Our results demonstrate that it interferes either as an agonist or antagonist with the two types of α-adrenoceptors in the rat vas deferens. In the stimulated prostatic half of rat vas deferens, St-587 increased the height of the twitch response probably through a mechanism where postsynaptic α_1-adrenoceptors are involved. This effect was increased in the presence of yohimbine (0·3 μM) and was not reduced in reserpinized animals, suggesting that it is unrelated to the functioning of adrenergic nerve terminals. On the other hand, prazosin antagonized this enhancing effect in a competitive manner giving the same pA_2 value as that obtained for the contractile effects of phenylephrine in the epididymal half. This enhancing effect has also been observed with other α_1-adrenoceptor agonists, like amidephrine (Butler & Jenkinson 1978) and cirazoline (Docherty & McGrath 1984) and in both cases α_1-adrenoceptor antagonists inhibited this enhancing effect. Thus it seems clear that postjunctional α_1-adrenoceptors are implicated in the potentiation produced by St-587 of the responses induced by field stimulation in the prostatic half of rat vas deferens.

In the presence of prazosin (0·1 μM), St-587 behaved as a partial agonist of presynaptic α_2-adrenoceptors in the prostatic half of rat vas deferens. Firstly, it partially inhibited, in a concentration-dependent manner, the twitch response induced by field stimulation. These inhibitory effects and those induced by clonidine, were completely reversed by the highly selective α_2-adrenoceptor antagonist, yohimbine (Doxey et al 1984), in the same range of concentrations. Thus, it appears that the presynaptic inhibitory effects induced by St-587 in the prostatic portion of rat vas deferens are mediated by its interaction with α_2-adrenoceptors. Secondly, it antagonized the inhibition by clonidine of twitch responses, suggesting an antagonism of presynaptic α_2-adrenoceptors. This finding is compatible with the observation that St-587 antagonizes the inhibitory effects mediated by activation of presynaptic α_2-adrenoceptors on the electrically-induced heart rate increase in the pithed rat (Pichler & Kobinger 1985).

St-587 was unable to induce contractile effects in the unstimulated prostatic half of rat vas deferens. However, it competitively antagonized the concentration-response curves to phenylephrine, a selective agonist of α_1-adrenoceptors (Kobinger & Pichler 1981). These results indicate that it behaves as an α_1-adrenoceptor antagonist in the prostatic portion of rat vas deferens. However, this α_1-adrenoceptor antagonist activity does not cancel the potentiation of twitch responses induced in the prostatic half by St-587 via activation of α_1-adrenoceptors. Thus it seems that two different subpopulations of α_1-adrenoceptors may be implicated in these results. Recently, the existence of two pharmacologically distinct α_1-adrenoceptors subtypes in rat vas deferens and other tissues has been demonstrated. Both subtypes can mediate functional responses to noradrenaline and in binding studies they are not discriminated by prazosin but they are by phentolamine (Morrow & Creese 1986; Han et al 1987). St-587 could act on both prostatic α_1-adrenoceptors with a different affinity.

In contrast St-587 is a partial agonist of α_1-adrenoceptors in the unstimulated epididymal half. Firstly, the maximal

Table 1. Mean pD_2 values of phenylephrine and St-587 on the postsynaptic α_1-adrenoceptors in the epididymal half of rat vas deferens.

	a	b	c
Agonist	n	$pD_2 \pm$ s.e.m.	% $E_{max} \pm$ s.e.m.
Phenylephrine	6	5·84 ± 0·02	107 ± 2·4
St-587	6	5·88 ± 0·02	50 ± 1·2

a number of experiments.
b mean and standard error of the mean.
c maximal contractile response relative to 100 μM noradrenaline.

Table 2. Mean pA_2 values of prazosin against phenylephrine and St-587 in the epididymal half of rat vas deferens.

	a	b	c
Agonist	n	$pA_2 \pm$ s.e.m.	slope ± s.e.m.
Phenylephrine	6	8·25 ± 0·03	1·01 ± 0·08
St-587	6	8·48 ± 0·12	0·92 ± 0·05

a number of experiments.
b mean and standard error of the mean.
c slope of Schild plots.

effect it elicited is less than the maximum contractile response to phenylephrine. Secondly, prazosin shows the same pA_2 against both agonists. This partial agonist activity of St-587 has also been detected in other isolated preparations such as the guinea-pig aorta (Beckeringh et al 1984). The different action of St-587 on both unstimulated portions of rat vas deferens could be explained as a distinct efficiency stimulus-response mechanism along the tissue after α_1-adrenoceptor activation.

It can be concluded that St-587 shows a broad spectrum of activity on α-adrenoceptors of rat vas deferens and caution is necessary when it is used as a pharmacological tool in the study of these receptors. In addition our results confirm that the sensitivity of the rat vas deferens to α-adrenoceptor agonists can depend on that portion of the preparation used.

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