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Pre- and postsynaptic effects of sulpiride in the rat isolated vas deferens

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It is known that many sympathetic nerve terminals have not only α-adrenoceptors but also dopamine receptors, that regulate the stimulation-evoked release of noradrenaline by means of a negative feed-back mechanism (Enero & Langer 1975; Hope et al 1978; Dubocovich & Langer 1980). In the rat vas deferens, in addition to the classical postsynaptic α-adrenoceptors, the existence of presynaptic α-adrenoceptors (Drew 1977) and postsynaptic and presynaptic dopamine receptors has been described (Simon & Van Maanen 1976; Tayo 1977, 1979). On the other hand, it has been reported that some differences do exist between pre- and postsynaptic dopamine receptors (Goldberg et al 1978) as occurs with α-ardrenoceptors (Langer 1979). In order to obtain more information about the nature of the two types of dopamine receptors in the rat vas deferens, we have investigated the activity of several benzamides, known as potent dopamine antagonists (Jenner & Marsden 1979). This report describes the effects of sulpiride, a substituted benzamide, on pre- and postsynaptic α-adrenoceptors and dopamine receptors in the rat isolated vas deferens.

Male Wistar rats (300–325 g) were killed by cervical dislocation and exsanguination. Both vasa deferentia were removed and carefully cleaned. The whole vasa were set up in isolated organ baths containing 20 ml of Krebs solution, as modified by Huković (1961). The solution was maintained at 32 ± 0·5 °C and gassed with 95% O_2–5% CO_2. 1 h was allowed to elapse before starting the experiment. The organ responses against 0·5 g tension were recorded by means of an isotonic Ealing transducer on an Omniscribe pen-recorder.

Presynaptic studies. Platinum ring electrodes were placed above and below of the vas deferens and continuous field stimulation was carried out with an Ealing Stimulator (0·1 Hz, 3 ms and 20–30 V). When the twitch responses to field stimulation became stable, the agonists were added to the bath in a cumulative concentration schedule every 3–5 min. When the concentration-response curve with the agonist was obtained, a full recovery after washout was difficult to obtain and it was only possible to evaluate one concentration-response curve in each tissue. In order to resolve this problem, the following procedure was used. When a maximal inhibitory effect was obtained with the agonist, the initial twitch response was restored by the cumulative addition of sulpiride in a concentration-dependent manner. Using the method described by Davis et al (1980) for isolated bronchi, it was possible to determine at least three dose-ratios of agonist from the isoresponse points of the two curves obtained in each preparation, and the pA_2 and slopes of Arunlakshana & Schild (1959) were calculated.

Postsynaptic studies. Cumulative concentration-response curves of isotonic contractions were obtained in each vas deferens with the agonist. The concentration-response curves as control and in the presence of sulpiride ($3 \cdot 10^{-5}$, $1 \cdot 10^{-4}$ and $3 \cdot 10^{-4}$ mol litre^{-1} added 5 min before obtaining each curve) were constructed for each preparation. In this situation, it was possible to calculate the pA_2 and the slopes of the Schild plots (Arunlakshana & Schild 1959) in each experiment.

All the results are given as the mean ± s.e.m. The means were statistically compared using Student's *t*-test and the differences were significant when $P < 0{\cdot}05$.

Clonidine and apomorphine were used as presynaptic α-adrenergic and dopaminergic agonists respectively because they do not induce contractile effects at the concentrations used. Both agonists inhibited in a concentration dependent fashion the twitch contraction of the rat isolated vas deferens obtained with continuous field stimulation, and sulpiride restored this to the initial value after graded additions (Fig. 1). Calculating, by means of interpolation, the concentration of the agonist used as control equivalent to the restored response with sulpiride, at least three dose-ratios were determined in each experiment. With the calculated values, the Schild plots were constructed and the pA_2 and the slopes of the regression lines were obtained for the interaction of sulpiride with both agonists (Table 1). As can be seen, the two slopes were near the theoretical value of 1 and the differences between the two pA_2 values were not statistically significant.

Noradrenaline and dopamine were used as postsynaptic α-adrenergic and dopaminergic agonists respectively

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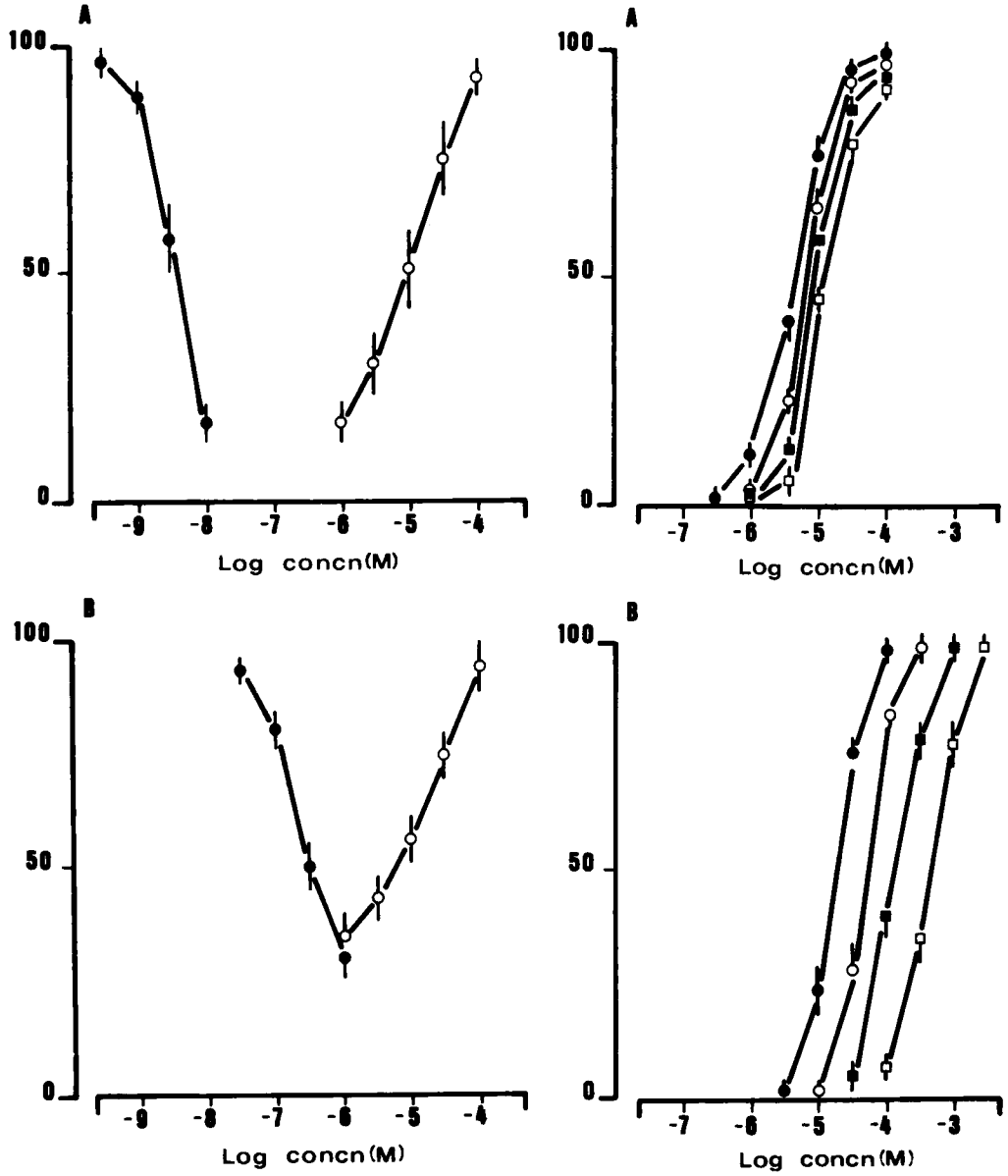

FIG. 1. (left-hand side) Concentration-response curves of inhibitory effects (●—●) of clonidine (A) and apomorphine (B) on twitch contractions of the rat vas deferens (0·1 Hz; 3 ms; 20–30 V) and their antagonism by sulpiride (○—○). The concentration-response curves with sulpiride were obtained with increasing addition of antagonist after the maximal inhibitory effect with agonist was achieved. Abcissa: 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. (right-hand side) Effects of sulpiride on the concentration-response curves of the contractile responses of noradrenaline (A) and dopamine (B) in the rat vas deferens. (●—●), control; (○—○), in the presence of 3×10^{-5} M sulpiride; (■—■), in the presence of 1×10^{-4} M sulpiride; (□—□), in the presence of 3×10^{-4} M sulpiride. Abscissa: logarithm of drug molar concentration. Ordinate: % of maximal control response. Each point is the mean of at least six experiments. Vertical bars show standard errors of the mean.

because they behave as full agonists in the rat vas deferens (Patil et al 1967). The concentration-response curves of dopamine were shifted to the right by increasing concentrations of sulpiride, but those of noradrenaline were not displaced (Fig. 2). The pA_2 of sulpiride against dopamine is also shown in Table 1.

Clonidine inhibits the twitch contraction of the stimulated rat vas deferens through the activation of presynap-

Table 1. Mean pA_2 and slopes of Schild plots (Arunlakshana & Schild, 1959) of sulpiride and tolazoline in the rat vas deferens.

Agonist	n[a]	Sulpiride $pA_2 \pm$ s.e.m.[b]	Sulpiride slope ± s.e.m.	n	Tolazoline $pA_2 \pm$ s.e.m.	Tolazoline slope ± s.e.m.
			Presynaptic			
Clonidine	6	5·28 ± 0·12	0·95 ± 0·03	6	6·33 ± 0·09	1·06 ± 0·03
Apomorphine	6	5·49 ± 0·10	0·93 ± 0·06	6	6·15 ± 0·08	1·01 ± 0·03
			Presynaptic			
Noradrenaline	6	<4·00	—		—	—
Dopamine	6	4·81 ± 0·03	1·02 ± 0·08		—	—

[a] Number of experiments.
[b] Standard errors of the mean.

tic α-adrenoceptors (Drew 1977; Doxey et al 1977). Furthermore, it has also been reported that apomorphine induces a presynaptic inhibition in the rat vas deferens by stimulating dopamine receptors that are located in the nerve terminals (Tayo 1977), in contrast with the observation that this type of receptor does not exist in the guinea-pig and mouse vas deferens (Bell & Matalanis 1977; Hurst et al 1979). However, our results suggest that both agonists activate the presynaptic α-adrenoceptors in the rat vas deferens. In fact, sulpiride, a dopamine receptor antagonist with low α-adrenergic blocking activity (Kohli & Cripe 1979) that selectively inhibits the presynaptic dopamine receptors in the cat spleen (Dubocovich & Langer 1980), competitively antagonized with the same potency the presynaptic inhibitory effect of both clonidine and apomorphine in the field stimulated rat vas deferens, as can be seen from the pA_2 values given in Table 1. Moreover, it has recently been demonstrated that metoclopramide, sulpiride and sultopride, three benzamide derivatives, have a preferential α-adrenergic presynaptic effect in the rat vas deferens (Spedding 1980). Thus, it appears that the presynaptic inhibition induced by apomorphine in the rat vas deferens is mediated by its interaction with α-adrenoceptors or that in this preparation the presynaptic dopamine receptors are different from those described in the cat spleen. We have observed, however, that tolazoline, a preferential presynaptic α-adrenergic antagonist (Borowski et al 1977), shows a similar activity against both agonists (Table 1) and this is not compatible with the existence of different presynaptic receptors for dopamine in the rat vas deferens.

Sulpiride competitively antagonized the postsynaptic effects of dopamine in the rat vas deferens without affecting the concentration-response curves of noradrenaline. Thus, it would appear that dopamine contracts the rat isolated vas deferens through the activation of specific postsynaptic dopamine receptors. These results are in agreement with those obtained by Simon & Van Maanen (1976) and Tayo (1979), who found evidence for these receptors in the vas deferens of the rat in contrast with the vas deferens of guinea-pig (Tayo 1979) and mouse (Gibson & Samini 1979). The calculated pA_2 of the interaction of sulpiride with dopamine is similar to that observed in the renal vascular bed of the rat (Schmidt & Imbs 1980). The low potency of sulpiride antagonizing the postsynaptic effect of dopamine, the activity of dopamine at micromolar concentrations, and the fact that apomorphine is a partial agonist (Simon & Van Maanen 1976), all suggest that the dopamine receptors that mediate the contractile responses of the rat vas deferens probably belong to the D_1 type of the classification proposed by Kebabian & Calne (1979).

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