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Prazosin-induced Blockade of Extraneuronal Uptake Facilitates Dopaminergic Modulation of Muscle Twitches in Rat Vas Deferens

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Abstract

Preliminary findings in our laboratory have shown that prazosin augmented the inhibitory effects of dopamine on the electrically-evoked muscle twitches in rat vas deferens. In this study, we opted to investigate the underlying mechanism and whether a prazosin-induced blockade of extraneuronal uptake process may be involved.

Cumulative additions of dopamine ($1{\cdot}8 \times 10^{-7}$–$4{\cdot}4 \times 10^{-5}$ M) elicited slight (< 30%) but dose-related inhibition of electrically-evoked (0·05 Hz, 1 ms duration and supramaximal voltage) muscle twitches of the vas deferens. Pretreatment with cocaine (10 μM), prazosin (50 nM) or oestradiol (10 μM) produced comparable potentiation of the inhibitory responses of dopamine; the pD_2 values to dopamine amounted to $4{\cdot}47 \pm 0{\cdot}20$, $4{\cdot}72 \pm 0{\cdot}21$ and $4{\cdot}56 \pm 0{\cdot}19$, respectively. A lower concentration of prazosin (5 nM) failed to alter dopaminergic responses. Further potentiation of dopamine responses was demonstrated in tissues preincubated with a combination of cocaine plus prazosin (50 nM), or cocaine plus oestradiol (pD_2, $5{\cdot}40 \pm 0{\cdot}11$ and $5{\cdot}42 \pm 0{\cdot}05$, respectively). However, a mixture of all three drugs failed to elicit any further increase in dopamine responses, a finding that may suggest an extraneuronal uptake blocking activity for prazosin. Inhibition of muscle twitches evoked by bromocriptine, a dopaminoceptor agonist which is not a substrate for extraneuronal uptake, was not affected by prazosin (50 nM) pretreatment.

The findings presented in this study emphasize the role of dopamine in modulating noradrenergic neurotransmission in rat vas deferens. More importantly, the results suggest that prazosin may act to block the extraneuronal uptake at noradrenergic sites, an effect that may account for its capability to facilitate dopaminergic modulation of noradrenergic neurotransmission.

The main route for inactivation of catecholamines is by active uptake into the nerve terminals, a process referred to as neuronal uptake ($uptake_1$) (Iversen 1974; Baumann & Koella 1980; Burgen & Mitchell 1985). A second route whereby catecholamines are removed from the synaptic cleft is into non-neuronal tissues (extraneuronal uptake or $uptake_2$) such as vascular and non-vascular smooth muscles, cardiac muscles and certain glandular tissues (Gillespie 1973; Iversen 1973; Grohmann & Trendelenburg 1984). Extraneuronal uptake is of little physiological significance; only under conditions of $uptake_1$ blockade may $uptake_2$ become the major inactivating mechanism (Langer 1970; Hughes 1972). Extraneuronal uptake is usually followed by metabolism of the transported catecholamines by the combined action of monoamine oxidase and catechol-*O*-methyl transferase (Gillespie 1973; Iversen 1973).

It is widely accepted that dopamine exerts inhibitory influences on noradrenergic neurotransmission in a variety of peripheral tissues (Dubocovich & Langer 1980; Dopico et al 1986; Carratu et al 1989; Norenberg & Illes 1989; Smith & Rowland 1989). Initial findings in our laboratory have shown that prazosin, an α_1-adrenoceptor antagonist, enhanced the presynaptic inhibitory effect of dopamine on electrically-evoked muscle twitches in rat vas deferens; the mechanism, however, is not clear. One possible mechanism may relate to the ability of prazosin to interrupt the extraneuronal elimination of dopamine. If this is true, then more quantities of dopamine would be available in the synaptic cleft in the vicinity of nerve terminals with the subsequent elicitation of greater presynaptic inhibition of noradrenergic transmission. Blockade of $uptake_2$ has been reported for some α-adrenergic blockers such as phenoxybenzamine (Lightman & Iversen 1969). In addition, prazosin has been shown to inhibit extraneuronal accumulation of radiolabelled isoprenaline in rat heart tissues (Akimoto et al 1989). The main objective of this study was, therefore, to investigate whether facilitation of dopaminergic modulation of neurotransmission by prazosin in rat vas deferens may be accounted for by a prazosin-induced blockade of $uptake_2$. To this end, experiments were undertaken to determine the effect of prazosin or the $uptake_2$ blocker, oestradiol, alone and in combination with cocaine, an $uptake_1$ blocker, on low-frequency muscle twitches in rat vas deferens. Since the results showed that pretreatment with either prazosin or oestradiol produced similar potentiation of dopamine responses and their effects were not additive, further experiments were designed to determine whether the effect of prazosin is dose-dependent, and the influence of prazosin on inhibitory responses to dopaminergic agonists that are not substrates for $uptake_2$, e.g. bromocriptine (Lieberman & Goldstein 1985).

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Materials and Methods

The method described by Tayo (1981) and Leedham & Pennefather (1982) for isolation and electrical stimulation

of rat vas deferens was employed. Briefly, male Wistar rats (180–220 g, High Institute of Public Health, Alexandria, Egypt) were killed by a blow on the head and the vasa were dissected out. Each vas was bisected transversely and the prostatic portion was suspended between a pair of platinum plate electrodes under 1-g tension in Krebs solution of the following composition (mM): NaCl 120·0, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4.7H_2O$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25, glucose 5·6. The solution was gassed with 5% CO_2 in O_2 and maintained at 37°C. The upper end of the vas was attached to a Grass force displacement transducer (FT03C) connected to a Grass polygraph (7D). The tissues were allowed to equilibrate for 60 min during which the bathing solution was replaced every 10 min. The vas was field-stimulated with pulses of 1 ms duration delivered at 0·05 Hz at supramaximal voltage (45–55 V) using a Grass stimulator (S48).

Experimental protocols

Influence of prazosin on inhibitory effects of dopamine on muscle twitches. This experiment investigated the effect of cocaine (10 μM) alone or in combination with prazosin (5 and 50 nM) on the inhibitory effects of dopamine on the field-stimulation muscle twitches in rat vas deferens. After a preliminary equilibration period of 60 min and twitch stabilization, a cumulative concentration-response curve for dopamine ($1{\cdot}8 \times 10^{-7}$–$4{\cdot}4 \times 10^{-5}$ M) was established. Each dose was added when the response to the previous one had reached a plateau. Drugs (cocaine and prazosin) tested for their effects on dopamine responses were added to the bathing solution 30 min earlier.

Influence of oestradiol on inhibitory effects of dopamine on muscle twitches. This experiment tested the possibility that prazosin augmented the inhibitory effects of dopamine on electrically-evoked muscle twitches in rat isolated vas deferens via blockade of the extraneuronal uptake process. Percentage changes in the amplitude of the electrically-evoked muscle twitches elicited by dopamine was assessed in tissues pretreated with prazosin (50 nM), oestradiol (10 μM), oestradiol and cocaine, prazosin and cocaine, or a combination of oestradiol, prazosin and cocaine. The concentrations of cocaine and oestradiol employed in the present study have been proved adequate for blocking the neuronal and extraneuronal uptake processes, respectively (El-Mas et al 1989; El-Mas & Hughes 1990).

Influence of prazosin on inhibitory effects of bromocriptine on muscle twitches. The ability of prazosin to alter inhibitory responses evoked by dopaminergic agonists that are not eliminated by uptake$_2$ such as bromocriptine (Lieberman & Goldstein 1985) was investigated in this experiment. A series of concentrations of bromocriptine (1×10^{-7}–8×10^{-6} M) were added in a cumulative manner and percentage changes in the amplitude of the twitch response were evaluated in the absence and in the presence of prazosin (50 nM).

Drugs

Drugs were supplied as follows: dopamine hydrochloride (3-hydroxytryptamine, Sigma), cocaine hydrochloride (Boots), 17-β-oestradiol (Sigma), bromocriptine methansulphonate (Sandoz), prazosin hydrochloride (Sigma). A fresh solution of dopamine was made daily in a catecholamine diluent of the following composition (mM): NaCl 154, NaH_2PO_4 1·2, ascorbic acid 0·23. Cocaine was dissolved in distilled water and oestradiol in ethanol. A stock solution of prazosin was prepared by levigation with a drop of glycerol and then a 5% dextrose solution was added slowly whilst stirring vigorously and stored at 4°C. Stock solutions of bromocriptine were prepared in 1% lactic acid.

Analysis of data

Values are presented as mean ± s.e.m. Responses (twitch inhibition) were expressed as a percentage change of the basal twitch amplitude. The potencies of the agonists (dopamine or bromocriptine) were expressed as pD_2 values which are the negative logarithm of the molar concentration producing 50% inhibition (-log IC50) of the twitch response (Leedham & Pennefather 1982). The IC50 was estimated for individual experiments by linear regression of the linear portions (approx. 15–85% range) of the dose response curves (Burnstock & Meghji 1981). One-way analysis of variance followed by the Bonferroni modified post-test (Wallenstein et al 1980) was used for multiple comparisons among means. The criterion for statistical significance was set at the 0·05 level.

Results

Transmural stimulation of the prostatic portion of the rat vas deferens with pulses of 1 ms duration at 0·05 Hz and supramaximal voltage produced twitches which reached a steady state within 10 min. The twitches were neurogenic in origin, as they were totally abolished by guanethidine (10 μM) (data not shown).

Cumulative additions of dopamine ($1{\cdot}8 \times 10^{-7}$–$4{\cdot}4 \times 10^{-5}$ M) slightly reduced the amplitude of the twitches; the highest inhibition amounted to approximately 30% (Fig. 1). Neuronal uptake blockade by cocaine (10 μM) significantly ($P < 0{\cdot}05$) enhanced the inhibitory effects of dopamine as indicated by the upward shift of the concentration-effect curves (Fig. 1). Combined pretreatment of the tissues with cocaine and prazosin resulted in further augmentation

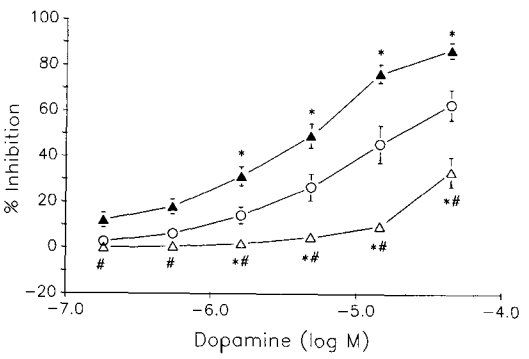

FIG. 1. Inhibitory effects of dopamine on the electrically-evoked (0·05 Hz, 1 ms duration and supramaximal voltage) muscle twitches in rat isolated vas deferens. Dopamine was tested alone (Δ, n = 6) or in tissues pretreated with cocaine (10 μM) alone (○, n = 6) or combined with prazosin (50 nM) (▲, n = 9). Responses are expressed as a percentage inhibition of the basal twitch amplitude. *$P < 0{\cdot}05$ vs cocaine, #$P < 0{\cdot}05$ vs cocaine plus prazosin.

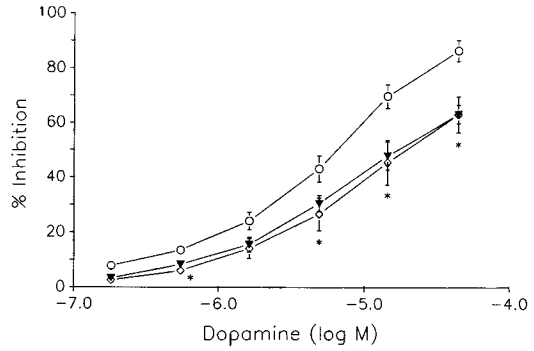

FIG. 2. Inhibitory effects of dopamine on the electrically-evoked (0·05 Hz, 1 ms duration and supramaximal voltage) muscle twitches in rat isolated vasa deferentia pretreated with cocaine (10 μM) alone (◇, n = 6) or in combination with different concentrations of prazosin 5 (▽, n = 8) and 50 nM (○, n = 7). Note that only the higher concentration of prazosin enhances dopamine responses. Responses are expressed as a percentage inhibition of the basal twitch amplitude. *$P < 0.05$ vs cocaine plus prazosin (50 nM).

of the inhibitory effects of dopamine (Fig. 1). Regression analysis of the linear portions of the curves revealed that the pD_2 value of dopamine in tissues that had received the combined pretreatment was significantly ($P < 0.05$) higher compared with the corresponding value of cocaine-pretreated tissues (5·40 ± 0·11 and 4·47 ± 0·20, respectively). The ability of prazosin to augment dopamine responses appeared to be concentration-dependent since the use of a lower concentration of prazosin (5 nM) along with cocaine failed to elicit any further increase in the inhibitory responses to dopamine (Fig. 2). In all cases, the correlation coefficients of the regression lines were highly significant and ranged from 0·94 to 0.98.

In tissues pretreated with either prazosin (50 nM) or oestradiol (an uptake$_2$ blocker, 10 μM), dopamine elicited identical inhibitory effects on muscle twitches (Fig. 3). The pD_2 values of dopamine with these single pretreatments were similar to the corresponding value obtained in tissues that were pretreated with cocaine alone (Table 1). Fig. 4 depicts the inhibitory responses to dopamine on muscle twitches in tissues receiving combined pretreatment with cocaine and prazosin, cocaine and oestradiol, or a mixture of the three drugs. The inhibitory responses as well as the

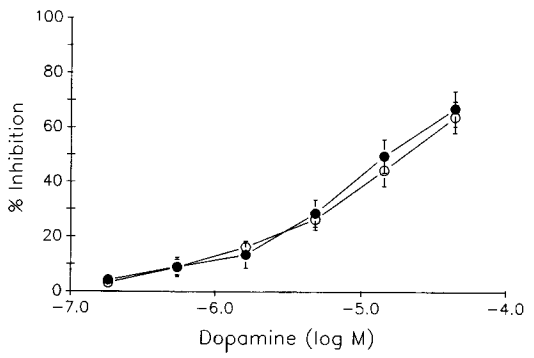

FIG. 3. Inhibitory effects of dopamine on the electrically-evoked (0·05 Hz, 1 ms duration and supramaximal voltage) muscle twitches in rat isolated vasa deferentia pretreated with prazosin (50 nM) (●, n = 7) or oestradiol (10 μM) (○, n = 8). Responses are expressed as a percentage inhibition of the basal twitch amplitude.

Table 1. The pD_2 values for dopamine at presynaptic receptors in the rat isolated vas deferens pretreated with cocaine (10 μM), prazosin (50 nM), oestradiol (10 μM) and their combinations.

Pretreatment	n	pD_2
Cocaine	6	4·47 ± 0·20
Prazosin	7	4·72 ± 0·21
Oestradiol	8	4·56 ± 0·19
Cocaine + prazosin	9	5·40 ± 0·11*
Cocaine + oestradiol	8	5·42 ± 0·05*
Cocaine + prazosin + oestradiol	9	5·51 ± 0·05*

Values are mean ± s.e. *$P < 0.05$ vs individual treatments.

pD_2 values for dopamine among the three groups were not statistically different (Fig. 4, Table 1).

Cumulative addition of bromocriptine (1×10^{-7}–8×10^{-6} M) caused a concentration-dependent reduction in the amplitude of the electrically-evoked muscle twitches in rat isolated vas deferens (Fig. 5). These responses to bromocriptine were not altered in tissues pretreated with prazosin (50 nM) (Fig. 5).

Discussion

The main finding of the current study is that prazosin facilitates the dopaminergic modulation of neurotransmission in rat isolated vas deferens through a mechanism that may involve, at least in part, blockade of the extraneuronal uptake process. This view is supported by the notion that the dopamine-induced reductions of the electrically-evoked muscle twitches were equally potentiated in tissues pretreated with prazosin and oestradiol, and the effects of the two drugs were not additive. Further, the same dose of prazosin did not alter the inhibitory effects of bromocriptine, a dopaminergic agonist that is not a substrate for uptake$_2$ (Lieberman & Goldstein 1985). Blockade of uptake$_2$ and hence potentiation of dopamine responses, appears to be dependent on the concentration of prazosin employed.

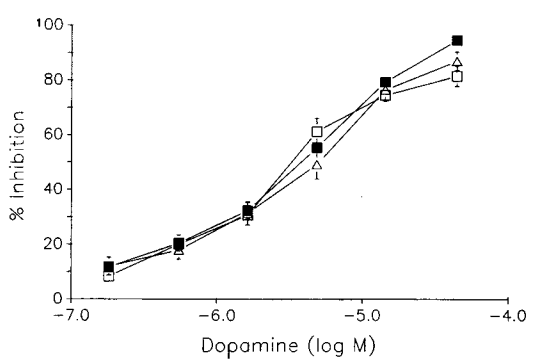

FIG. 4. Inhibitory effects of dopamine on the electrically-evoked (0·05 Hz, 1 ms duration and supramaximal voltage) muscle twitches in rat isolated vasa deferentia pretreated with various combinations of oestradiol (10 μM), prazosin (50 nM) and cocaine (10 μM). Note that the use of a mixture of prazosin and oestradiol, in addition to cocaine, elicits no further increases in dopamine responses compared with each of the two drugs when used alone. Responses are expressed as a percentage inhibition of the basal twitch amplitude. Dopamine + cocaine + prazosin △ (n = 9), dopamine + cocaine + oestradiol □ (n = 8), dopamine + cocaine + prazosin + oestradiol ■ (n = 9).

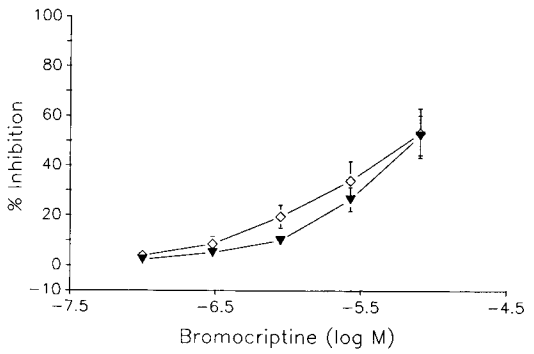

FIG. 5. Inhibitory effects of cumulative additions of bromocriptine on the electrically-evoked (0·05 Hz, 1 ms duration and supramaximal voltage) muscle twitches in rat isolated vasa deferentia in absence (◇, n = 5) and presence of prazosin (50 nM) (▽, n = 6). Responses are expressed as a percentage inhibition of the basal twitch amplitude.

The initial impetus of the present study was the interesting finding that prazosin, an α_1-adrenergic antagonist, potentiated the inhibitory effects of dopamine on electrically-evoked muscle twitches in rat vas deferens. We opted, therefore, to investigate the underlying mechanism and whether it could involve blockade of the extraneuronal uptake process. It is notable that the bulk of the reports, including ours, that have studied the regulation of noradrenergic neurotransmission, involved measurement of transmitter overflow from tissues pre-incubated with radiolabelled neurotransmitters (El-Mas et al 1989; Molderings et al 1989; El-Mas & Hughes 1990). Others (Doxey et al 1977; Tayo 1981; Dopico et al 1986) have employed low-frequency transmural stimulation as an alternative method of studying prejunctional modulation of neurotransmission. The latter method was utilized in the present study for assessment of presynaptic modulation of neurotransmission in rat vas deferens.

The current study presented a number of observations that implicate blockade of extraneuronal uptake process as the mechanism that may explain the capability of prazosin to facilitate dopaminergic modulation of neurotransmission in rat vas deferens. First, the use of either prazosin or oestradiol markedly potentiated the inhibitory responses of dopamine to comparable levels and so were the effects of their combinations with cocaine. This is confirmed by comparison of the pD_2 values derived from regression analysis of the concentration-response curves of dopamine established under the different experimental settings. Second, in cocaine-pretreated tissues, the use of a mixture of prazosin and oestradiol potentiated dopamine responses to levels that were not statistically different from those of separate treatments, a finding that may suggest one and single mechanism, possibly blockade of uptake$_2$, for the two drugs in the potentiation of dopamine effects. Third, if prazosin exerts its potentiating effect by preventing the uptake of dopamine into extraneuronal sites, then it should not alter inhibitory responses evoked by dopaminergic agonists, e.g. bromocriptine in the current study, for which uptake$_2$ is not a considered site of loss. Taken together, the aforementioned findings support our assumption that prazosin owes its ability to facilitate dopaminergic modulation of neurotransmission to interruption of the extraneuronal transport of dopamine.

The observation in the present study that blockade of neuronal (by cocaine) and extraneuronal (by oestradiol) processes potentiated dopamine responses to similar extents (see Table 1 for the pD_2 values) deserves a comment. It may simply suggest that the two processes contribute equally to dopamine elimination from the synaptic cleft in rat vas deferens. This assumption seems to contradict previous reports that have emphasized the importance of neuronal uptake as the principal inactivating mechanism for dopamine (Iversen 1973; Burgen & Mitchell 1985). Nevertheless, the issue of the relative importance of neuronal vs extraneuronal uptake is controversial and may vary with the density of sympathetic innervation and tissue type (Gillespie & Muir 1970; Gillespie 1973; Iversen 1973). Moreover, the exogenous addition of dopamine may have allowed greater proportions of the amine to reach the extraneuronal sites as has been suggested by Brandao et al (1980).

Results of the current and previous (Lightman & Iversen 1969; Akimoto et al 1989) studies may raise a question regarding the relationship between α-adrenoceptors and the uptake$_2$ process. It has been suggested that smooth muscle uptake might be related to α-adrenoceptors either as a step in gaining access to these receptors (Eisenfeld et al 1967), or because receptor binding is followed by cellular transport as a means of maintaining receptor availability for further drug interaction (Gillespie & Hamilton 1966). It may be argued, however, that some well-known uptake$_2$ inhibitors (e.g. steroids) lack any effect on α-adrenoceptors.

In conclusion, the present data suggest that a prazosin-induced blockade of the extraneuronal uptake process may account for its ability to enhance dopaminergic modulation of muscle twitches evoked by transmural nerve stimulation in rat isolated vas deferens. Radiometric and histochemical studies are needed, however, to support our hypothesis.

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