J. Pharm. Pharmacol. 1982, 34: 331–333
Communicated October 8, 1981

0022–3573/82/050331–03 $02.50/0

Phentolamine inhibition of rat seminal vesicle response to dopamine-mimetic drugs: α-adrenoceptor implication or lack of specificity?

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Rat isolated seminal vesicles are made to contract by catecholamines (Leitch et al 1954) and have been assumed to contain mainly α-adrenoceptors (Patil & Ruffolo 1980). Since previous studies (Kohli 1969; Simon & Van Maanen 1971; Cohen & Berkowitz 1975; Besse & Furchgott 1976; Bevan et al 1979; Baggio et al 1981), have shown that some pharmacological effects of dopamine(DA)-receptor agonists may be produced through α-adrenoceptor rather than DA-receptor activation, we have investigated the motor response of this preparation to noradrenaline, DA, apomorphine and (3,4-dihydroxyphenylamino)-2-imidazoline(DPI). Apomorphine and DPI are generally considered as selective agonists on brain excitatory-(DAe) and inhibitory-DA (DAi) receptors respectively (Struyker Boudier et al 1975; Cools et al 1976), DA acting indiscriminately on both.

Materials and methods

Rat isolated seminal vesicle. Adults rats (280–350 g) were killed by cervical dislocation, and the left seminal vesicle freed from the coagulation glands and extraneous tissue, removed by transection proximally at its point of entrance into the ductus deferens. A 30 ml organ bath, maintained at 37 ± 0·1 °C was used, filled with a Ringer-Locke solution of the following composition (g litre^{-1}): NaCl: 9·00, KCl: 0·42; $CaCl_2.2H_2O$: 0·24; $MgCl_2.6H_2O$: 0·005; $NaHCO_3$: 0·50; glucose: 0·50.

The bath solution was aerated with 95% + 5% (O_2 + CO_2) and was renewed every 5 min. Cumulative dose-effect curves were obtained as described by van Rossum (1965) with minor modifications. Resting tension was set at 0·7 g. In brief, after a 40 min stabilization, 1 ml of the bath solution was replaced by 1 ml of medium containing the agonist, this operation was repeated with progressively more concentrated solutions as soon as the response to the last dose reached its maximum and until the increase in drug concentration did not evoke any further response. Tension developed by the seminal vesicle was recorded by means of an appropriate isometric force transducer (Basile, Comerio, Italy; Cat. 7004). On the graph the maximum tension produced was considered 100 and the percentage of the response for each sub-maximal concentration was calculated. pD_2 values and relative affinity were then calculated.

To evaluate phentolamine-, haloperidol- and sulpiride-antagonism towards NA, DA and DPI, 1 ml of a solution of each antagonist replaced 1 ml of the bath-medium and 3 min after, without any washing, an agonist solution was added. The agonist was applied to the minimum dose producing the maximum contractile response.

* Correspondence

Drugs used

(−)-Noradrenaline tartrate monohydrate and (−)-dopamine HCl were from Merck (Milan, Italy), DPI HCl was a generous gift from Wander (Berne, Switzerland), phentolamine HCl and angiotensinamide II were from Ciba-Geigy (Basel, Switzerland), haloperidol HCl was from Luso Farmaco (Milan, Italy), carbamylcholine chloride was from Aldrich (Wisconsin, USA), apomorphine HCl and bradykinin were from Sandoz (Basel, Switzerland), $BaCl_2$ was from Carlo Erba (Milan, Italy) and (±)-sulpiride was from Ravizza (Milan, Italy).

Concentrations of drugs in saline form refer to the weight of the salt.

Drugs were dissolved at proper concentration in Ringer-Locke solution kept at 37 °C.

Results are expressed as arithmetical means (±s.e.).

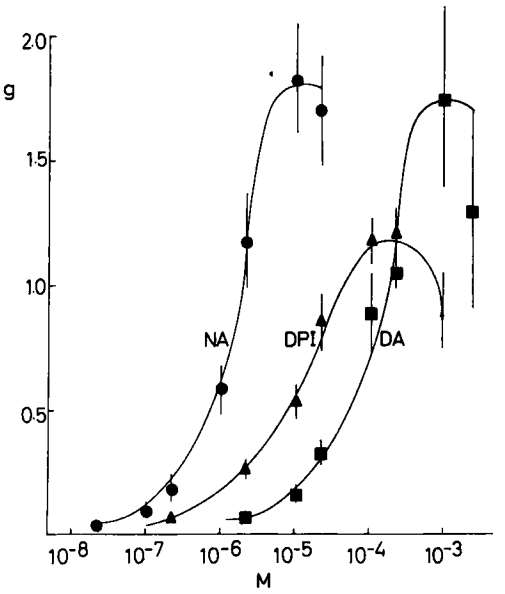

FIG. 1. Cumulative curves of dose-response of the biological preparation to: noradrenaline (NA): ●—●; (3,4-dihydroxyphenylamino)-2-imidazoline (DPI): ▲—▲; dopamine (DA): ■—■. Tension developed is expressed in g (ordinate).

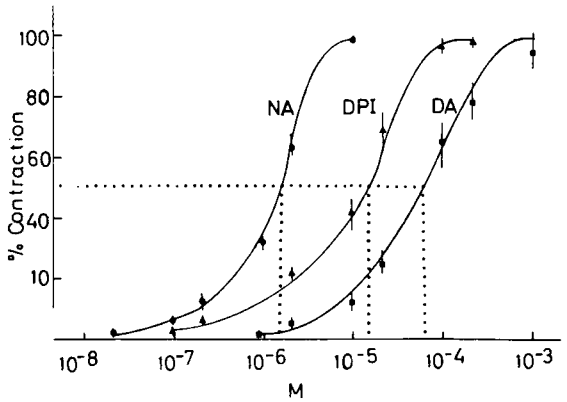

FIG. 2. Cumulative curves of dose-response of the biological preparation to: noradrenaline (NA): ●—●; (3,4-dihydroxyphenylamino)-2-imidazoline (DPI): ▲—▲; dopamine (DA): ■—■. Responses are expressed as percentage of the maximum response. Each point represents the mean from eight experiments. pD_2 was 5·80, 4·82 and 4·23 for NA, DPI and DA, respectively.

Results

Contractile responses to agonists. NA, DA and DPI as well as carbamylcholine and $BaCl_2$, evoked contractile responses, whereas apomorphine, angiotensinamide II and bradykinin in doses up to 1×10^{-3}, 1×10^{-4} and 1×10^{-4} M respectively, did not. Fig. 1 shows the cumulative dose-response curves for NA, DA and DPI (mean values for 8 dose-response curves on at least 4 biological preparations). The intrinsic activity of agonists evaluated according to van Rossum (1965) (Fig. 2) ranked NA > DA > DPI (1, 0·96 and 0·66 respectively), relative affinity ranked NA > DPI > DA (pD_2: 5·80, 4·82 and 4·23 respectively). However, DA and DPI responses differed qualitatively from that evoked by NA, in that responses evoked by DA and DPI at the highest doses were phasic but the response evoked by NA was not.

Interference of antagonists. To evaluate the interference of phentolamine, haloperidol and sulpiride on the contractile effects of NA, DA and DPI, the lowest maximum effective dose of each agonist was applied to the seminal vesicles pretreated with increasing concentrations of the antagonist. Fig. 3 shows a typical experiment for phentolamine-NA antagonism, inhibition being calculated by considering as 100 the contraction in the absence of the inhibitor.

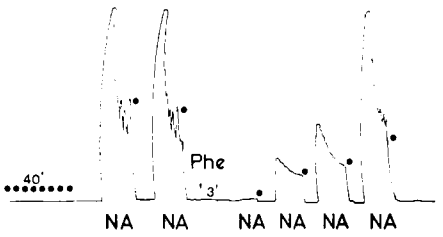

FIG. 3. Phentolamine (Phe) inhibition of rat seminal vesicle response to noradrenaline (NA). Seminal vesicles were equilibrated for 40 min, then NA (3×10^{-5} M) was applied, followed by wash (●). Phentolamine (Phe: 1×10^{-6} M) was in contact with the preparation for 3 min. Thereafter NA application followed by wash was repeated every 15 min until the contractile response reverted to that obtained before Phe application.

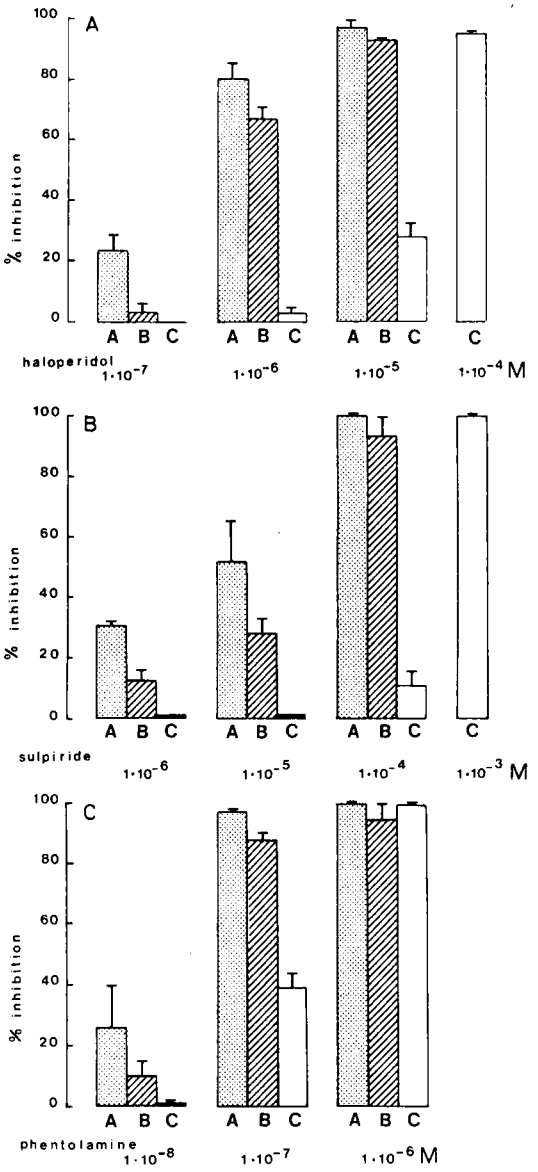

FIG. 4. Antagonism of contractile response of the rat isolated seminal vesicle to: dopamine (DA) (A: 1×10^{-4} M); (3,4-dihydroxyphenylamino)-2-imidazoline (DPI) (B: 3×10^{-5} M); noradrenaline (C: 3×10^{-5} M) by means of (A) haloperidol, (B) sulpiride, (C) phentolamine. Ordinate: percentage of response inhibition. Each value is the mean ± s.e. from 4–6 experiments.

Fig. 4 A–C show that not only haloperidol and sulpiride, but also phentolamine antagonized DA and DPI more

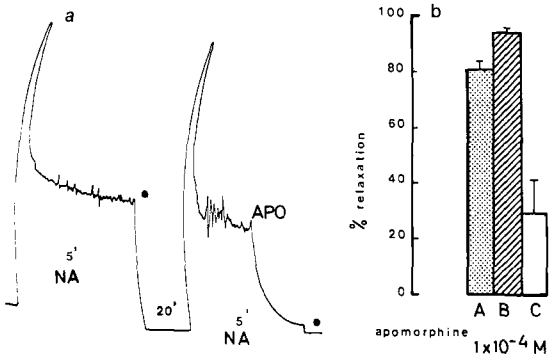

FIG. 5. Apomorphine (APO)-induced relaxing effect in the rat isolated seminal vesicle brought in contraction by noradrenaline (NA), dopamine (DA) and (3,4-dihydroxy phenylamino)-2-imidazoline (DPI). a: Agonist application (NA: 3×10^{-5} M) produced a rapid peak contraction followed by a slowly decreasing contraction phase. APO (1×10^{-3} M) was applied during the sustained contraction phase. b: Histogram height indicates the relaxing effect of APO (1×10^{-4} M) in the preparation made to contract by DA (A: 1×10^{-4} M), DPI (B: 3×10^{-5} M) and NA (C: 3×10^{-5} M). Each value is the mean from 4 experiments.

strongly than they did NA, sulpiride exhibiting the maximum and phentolamine the minimum of selectivity. On the other hand, phentolamine was the most and sulpiride the least potent antagonist.

Apomorphine relaxing effect. There is evidence that apomorphine possesses both agonist and antagonist properties on DA-receptors (Göthert et al 1977; Simon & Van Maanen 1971), therefore we wondered whether it antagonized DA, DPI and NA in the rat vesicle contraction test. In these experiments a single dose of the selected agonist was applied to make the preparation contract. The response exhibited a quick peak contraction followed by a slowly decreasing contraction phase (SDP) (Fig. 5–a). Apomorphine (1×10^{-4} M) added during the SDP which application of NA, DA and DPI relaxed the vesicle by 29, 81 and 94%, respectively (Fig. 5–b). On the other hand, neither the carbamylcholine- (1×10^{-5} M) nor $BaCl_2$- (1×10^{-3} M) induced contraction was affected by apomorphine.

Discussion

Two results deserve particular consideration. The first is the responsiveness of the rat isolated seminal vesicle to drugs considered to act primarily on DA-receptors. This implies that either DA receptors, besides α-adrenoceptors, are present in rat seminal vesicles or that, as already suggested (van Rossum 1965), there is 'a scale of α-adrenoceptors with at one end receptors which are best fitted by NA and at the other end receptors which are best fitted by DA'. Another possibility is that in this preparation α-adrenoceptors and DA-receptors are integrated in a single receptor-complex.

The second interesting result is that the potency of phentolamine as a DA- and a DPI-antagonist is greater than as a NA-antagonist. This suggests that it may be classified as a DA-antagonist rather than as an α-adrenoceptor antagonist, at least in some pharmacological tests, since much the same conclusion was reached in studying the influence of phentolamine on the action exerted by NA, DA, DPI and apomorphine on the guinea-pig isolated heart (Baggio et al 1981).

Finally the present paper shows that also in the rat isolated seminal vesicle as well as in the renal vascular bed (Kohli et al 1978; Kohli & Cripe 1979) i) sulpiride is the most selective DA-antagonist and ii) DPI and DA can be clearly distinguished from apomorphine, which has a mechanism of action that remains unaffected by sulpiride.

The results presented emphasize (see, for example, Walton et al 1978) the importance of detailed evaluation of drug selectivity in a particular experimental model adopted to identify the type of receptor(s) involved in pharmacological and/or physiological events related to catecholaminergic mechanisms.

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