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Analysis of the effects of apomorphine and bulbocapnine in relation to the proposed dopamine receptor

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On rat isolated vas deferens, apomorphine was found to be 1/5th as active as dopamine. Reserpine or cocaine pretreatment failed to reduce the response to apomorphine. Low concentrations of apomorphine which do not cause contraction of the tissue, antagonize the effects of dopamine. The effects of dopamine were antagonized equally by phentolamine or apomorphine. On rabbit aortic strips 4×10^{-4}M apomorphine repeated at 45 min intervals induces pronounced tachyphylaxis. During the tachyphylactic period dopamine was not inhibited to a significantly greater extent than was phenylephrine. Bulbocapnine tested against dopamine and phenylephrine yielded identical pA_2 values of 6. Only at 10^{-5}M was bulbocapnine observed to produce a preferentially greater blockade of dopamine. Dopamine or isoprenaline-induced relaxations of aortic strips were not blocked by bulbocapnine. Dopamine and 3×10^{-6}M bulbocapnine increased the chronotropic effects in atria. Apomorphine and higher doses of bulbocapnine produced rate decelerating effects. On atria low doses of apomorphine were equally effective in reducing the effects of dopamine or histamine. These results are discussed in light of the proposal that the effects of apomorphine and bulbocapnine involve dopamine receptor interactions. In all three tissues there was no clear cut evidence of specific dopamine receptors.

In addition to its neurotransmission function (Hornykiewicz, 1966), dopamine has been postulated to exert effects centrally that are the result of an interaction with sites other than those classically described as α- and β- adrenoceptors. The existence of dopamine receptors gained increased support when Goldberg, Sonneville & McNay (1968) demonstrated that the vasodilator effects of dopamine on renal blood vessels could not be abolished by adrenoceptor blockers. Furthermore, drugs such as haloperidol, chlorpromazine and bulbocapnine were apparently able to selectively antagonize the action of dopamine (see reviews by Goldberg, 1972; Woodruff, 1971).

In both the central nervous system and in the periphery, apomorphine is believed to exert some of its effects by activating specific dopamine receptors (Ernst, 1965; van Rossum, 1966; Andén, Ruben & others, 1967; Goldberg & others, 1968). During studies on the influence of apomorphine (and related substances) on isolated tissues, sympathomimetic effects were observed. These actions were intriguing in view of the proposed dopamine-like interactions for these compounds. We have examined the spectrum of action of apomorphine and bulbocapnine on peripheral effectors.

METHODS

Isolated tissues from albino rats (290–450 g), rabbits (1·5 to 2·9 kg) and guinea-pigs (500–700 g) of either sex were suspended in a 10 ml tissue bath containing a physiological salt solution at 37·5° ± 0·5°. The composition of the solution (mM) was: NaCl, 118; KCl, 4·7; $CaCl_2(2H_2O)$, 2·5; $MgCl_2(6H_2O)$, 0·54; $NaHCO_3$, 25; NaH_2PO_4, 1; and glucose, 11. The salts were dissolved in double distilled demineralized water containing 10 μg ml^{-1} of ethylenediamine tetraacetic acid (EDTA) to prevent spontaneous oxidation of the unstable substances. The tissue equilibration time for rat vas deferens (300 mg tension), guinea-pig right atrium (200–300 mg tension), and rabbit aorta (4 g tension) was 30 min, 1 h and 2½ h, respectively. The drug-induced effects were recorded either *via* light isotonic levers on a kymograph or isotonic and isometric myographs on an oscillograph. Cumulative dose-response curves were obtained as described by van Rossum (1963).

Drugs. (−)-Apomorphine HCl (Merck), (+)-bulbocapnine HCl, (−)-cocaine HCl (Merck), dopamine HCl (Regis and Calbiochem), (−)-isoprenaline-(+)-bitartrate (Sterling Winthrop), phentolamine mesylate (Ciba), (−)-phenylephrine (Ganes) and reserpine (Ciba). All drug solutions were prepared on the day they were used. Solutions of apomorphine, dopamine, phenylephrine and isoprenaline were made in saline containing 0·1% sodium metabisulphite.

Rat vas deferens. Both dopamine and apomorphine were tested on the normal tissue. Only apomorphine was tested on tissues from reserpine-pretreated rats (5 mg kg^{-1}, i.p., 16–24 h) and with these tissues the maximum contraction to dopamine (10^{-3}M) after the highest dose of apomorphine was considered as 100%.

Since various agonists exhibit autoinhibition when administered in cumulative doses to this tissue, the antagonist studies were performed with fixed concentrations of agonists. Paired contralateral tissues were exposed to 10^{-4}M phenylephrine and the maximum contraction was considered to be 100%. A submaximum dose of phenylephrine (2×10^{-5}M) or dopamine (5×10^{-5}M) was then added, washed out and then repeated in the presence of apomorphine, 3×10^{-5}M (3 min incubation) on one vas and phentolamine 10^{-7} (3 min incubation) on the other.

Rabbit aorta. Spirally cut thoracic aortic strips were prepared according to Furchgott & Bhadrakom (1953). Two agonists were studied on paired tissues. Apomorphine tachyphylaxis was produced by three repeated doses (4×10^{-4}M) at 45 min intervals. The tissues were thoroughly washed (6 × 5 min) and a partial dose-response curve for dopamine or phenylephrine was obtained before and after the tachyphylaxis.

Both phenylephrine and dopamine were also tested before and after bulbocapnine (60 min incubation). Only partial dose-response curves were obtained before the antagonist and before the termination of the experiment a maximum contraction was obtained with the highest dose of phenylephrine. Since the absolute maximum contraction values in two series of experiments did not differ, phenylephrine and dopamine contractions were expressed as fractions of the maximum obtained with phenylephrine. Dose-ratios were calculated according to Arunlakshana & Schild (1959).

In another series of experiments, α-adrenoceptors were blocked by phentolamine, 10^{-5}M (30 min incubation). The vascular tone was increased by 2×10^{-6}M histamine and the agonist-induced relaxation studied. The preparation was washed and the drug-induced relaxation examined in the presence of 10^{-5}M phentolamine, histamine, and bulbocapnine—which was added 10 min before the agonists.

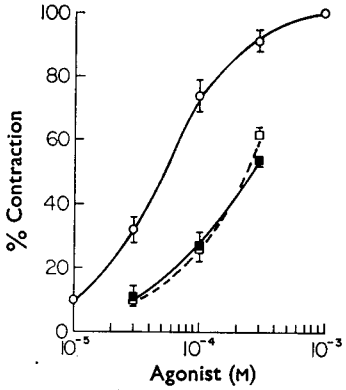

FIG. 1. Illustrates the dose-response curves of the rat isolated vas deferens to dopamine (○, n=6), apomorphine (■, n=10), and apomorphine after reserpine pretreatment (5 mg kg^{-1}, i.p., 16–24 h) (□, n=6). Maximum dopamine contraction=100%. Vertical lines are s.e.

Guinea-pig atrium. Cumulative dose-response curves for dopamine, apomorphine, bulbocapnine and histamine were constructed. The antagonism to submaximal doses of dopamine and histamine by bulbocapnine or apomorphine was studied. The rates were counted directly from the oscillograph tracings.

RESULTS

Effects of apomorphine on rat vas deferens. The dose response curves of dopamine and apomorphine are illustrated in Fig. 1. The solubility limitations of apomorphine made it impossible to test concentrations higher than 3×10^{-4}M. The negative log molar ED50 value for dopamine was 4·25, while for apomorphine the value was estimated to be 3·62. If compared at a point corresponding to the 50% response level, apomorphine has 1/5th the activity of dopamine. Pretreatment with reserpine did not alter the effects of apomorphine. Low concentrations of apomorphine which did not contract the tissue, antagonized the effect of dopamine. In five other replicate experiments, cocaine, 10^{-5}M (8 min incubation) failed to alter the effects of 3×10^{-4}M apomorphine.

The contractile effects of phenylephrine and dopamine were antagonized by phentolamine or apomorphine. Dopamine was more intensely affected by both phentolamine and apomorphine than was phenylephrine. However, both antagonists were equieffective against the same agonist ($P > 0{\cdot}05$). Thus, no distinction could be made between the dopamine-blocking effect of phentolamine or apomorphine. The results are illustrated in Fig. 2. The tissue sensitivity of the agonist can change with time (Furchgott, 1967; Patil, Hetey & others, 1970), hence time-control experiments with identical protocols without antagonists were made. In five tissues the contractile effect of a second dose of 2×10^{-5}M phenylephrine was increased by an average of 6·8% ± 2. On the other hand, the second dose of 5×10^{-5}M dopamine was desensitized by 17% ± 4. The data in Fig. 3 are corrected for the change in sensitivity of agonists during the experimental period.

Apomorphine tachyphylaxis on rabbit aorta. Since the rat vas deferens contains a dense adrenergic innervation that could obscure the drug-drug interactions, the testing was continued on the less densely innervated rabbit aorta. Apomorphine rapidly produced a tachyphylaxis in this preparation and the sensitivity to phenylephrine and dopamine was studied during the tachyphylactic period. At the highest dose of apomorphine (4×10^{-4}M) contraction of the aorta was only 20% of the

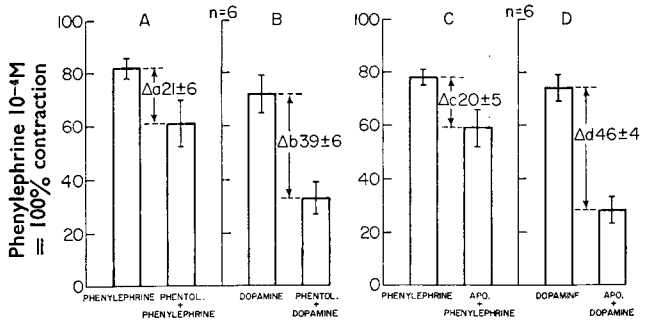

FIG. 2. Antagonism to phenylephrine (2×10^{-5}M) and dopamine (5×10^{-5}M) by phentolamine (10^{-7}M) and apomorphine (3×10^{-5}M) on the rat vas deferens. A and B or C and D were paired contralateral tissues. Δa and Δc or Δb and Δd were not significantly different ($P > 0.05$). Contact time for both antagonists was 3 min. On every tissue maximum response to 10^{-4}M phenylephrine = 100%.

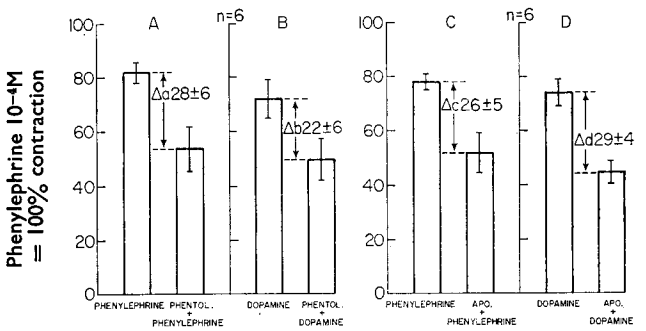

FIG. 3. Data from Fig. 2 are corrected for the change in sensitivity of the tissue during the experimental protocol. The effects of 2×10^{-5}M phenylephrine were increased by 7% and that of dopamine 5×10^{-5}M were desensitized by 17%. Δc and Δd are statistically insignificant $P > 0.05$).

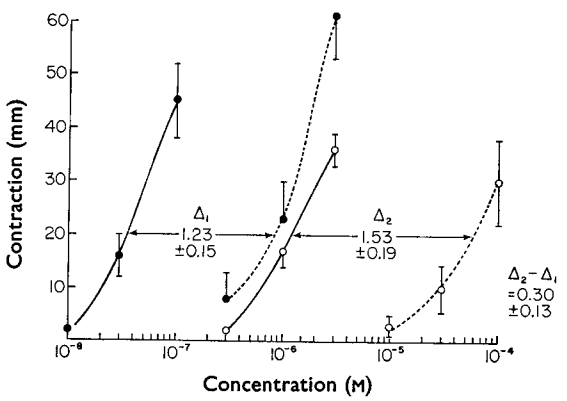

FIG. 4. Illustrates the sensitivity of the rabbit aorta to phenylephrine and dopamine before (— ● —, —○—) and after (- - ● - -, - - ○ - -) the tissue became tachyphylactic to 3 doses of 4×10^{-4}M apomorphine repeated at 45 min intervals. Both agonists were tested on 8 paired tissues. Shifts in the dose response curves ED20 mm are expressed in log units with s.e. values.

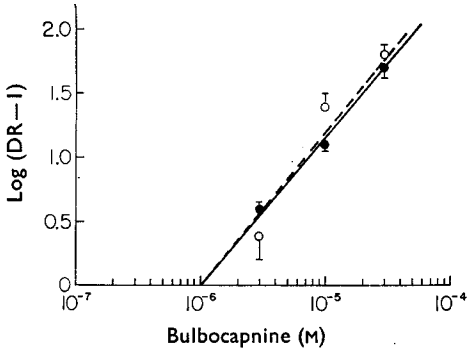

FIG. 5. pA_2 plots for bulbocapnine (60 min contact) against dopamine (○) and (−)-phenylephrine (●) on rabbit aortic strips (n=4–6). The lines are virtually superimposable. Both agonists were tested on paired tissues and each gave a pA_2 value of 6.

phenylephrine maximum. The second and third doses failed to elicit any response. Thus, when compared with tyramine tachyphylaxis (Patil & others, 1970), the rate of development of tolerance to apomorphine was very prompt. During the tachyphylactic period the dose response curves for both agonists were shifted to the right (Fig. 4). Phenylephrine was translated by 1·23 log units; dopamine by 1·53 log units. The difference in horizontal displacements between the two agonists was only 0·3 log units and was not significant ($P > 0{\cdot}05$). It should be emphasized that the time required for the construction of the dose-response curves of the agonists during apomorphine tachyphylaxis was long (45 to 60 min).

Bulbocapnine, 3×10^{-6}, 10^{-5}, and 3×10^{-5}M, caused a progressive parallel shift of the dose-response curves of phenylephrine and dopamine. Except at 10^{-5}M, bulbocapnine produced an equal degree of antagonism against the effects of phenylephrine or dopamine. The shift of the phenylephrine curve by 10^{-5}M bulbocapnine was $0{\cdot}16 \pm 0{\cdot}032$ log units, (n = 6). The same amount of bulbocapnine shifted the dopamine curve by $1{\cdot}40 \pm 0{\cdot}098$ log units (n = 6). The difference between the two shifts was significant ($P < 0{\cdot}05$). The pA_2 plots are illustrated in Fig. 5.

Although in cats and dogs, bulbocapnine is known to reduce vasodepressor effects of dopamine (Tseng & Walaszek, 1970; Goldberg & Musgrave, 1971), on the isolated aorta (after α-adrenoceptor blockade) both isoprenaline and dopamine produced relaxation which was not blocked by 10^{-5}M bulbocapnine. In fact, effects were slightly potentiated (Fig. 6).

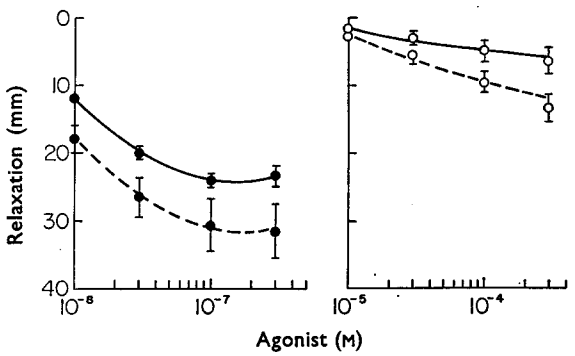

FIG. 6. Influence of bulbocapnine (10^{-5}M; contact time 10 min) on the relaxation of aortic strips induced by dopamine (○) and isoprenaline (●). The α-adrenoceptor was blocked by phentolamine and the tone was induced by histamine. Vertical lines are s.e. Broken lines = preparations with bulbocapnine.

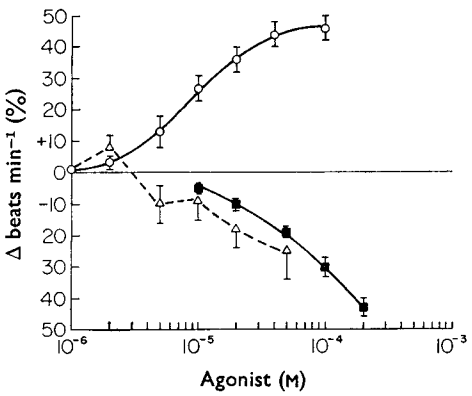

FIG. 7. Illustrates the dose response curves of agonists on guinea-pig right atria. Changes in rate are expressed as % variation from the initial basal beats min^{-1}. △, bulbocapnine (n=5–7). ■, apomorphine (n=6–32). ○, dopamine (n=4–14).

Guinea-pig atria. Dopamine produced significant chronotropic effects over the 10^{-4} to 10^{-6}M concentration range. Bulbocapnine, at 3×10^{-5}M, produced positive chronotropic effects but was depressant at all higher concentrations. At the concentrations studied, apomorphine was only depressant (Fig. 7). Concentrations of apomorphine which produce little or no chronotropic response failed to antagonize the effects of dopamine. At higher concentration the drug equally antagonized dopamine or histamine-induced effects (Table 1).

Table 1. *The mean reduction in chronotropic effects (with s.e.) of dopamine and histamine by apomorphine on guinea-pig atria.*

Drug and concn (M)	% Change in beats min^{-1} (+ increase − decrease)	n
Dopamine 1×10^{-5}	+ 29 ± 5	7
Apomorphine 5×10^{-5}	− 17 ± 2	9
Apomorphine 5×10^{-5} + dopamine* 1×10^{-5}	− 1 ± 3	9
Histamine 5×10^{-7}	+ 20 ± 3	7
Apomorphine 5×10^{-5}	− 17 ± 3	5
Apomorphine 5×10^{-5} + histamine* 5×10^{-7}	− 2 ± 5	5

* Added 5 min after apomorphine.

DISCUSSION

Since both reserpine pretreatment and cocaine failed to alter the effects of apomorphine on rat vasa deferentia the drug is presumed to act "directly" on receptor elements. Several lines of evidence already strengthened the notion that the drug acts directly on (dopamine) receptors in the central nervous system (Ernst, 1967; Pinder, Buxton & Green, 1971; Ungerstedt, 1971) and that the active pharmacophore may be the catecholamine moiety (Cannon, Smith & others, 1972; Burkman, 1973). Patil, La Pidus & others (1967) have demonstrated that catecholamines, including dopamine, tend to be principally direct-acting compounds.

During apomorphine tachyphylaxis in aortic tissue, no significant distinction could be made between the inhibition of phenylephrine and the inhibition of dopamine. Apomorphine may simply saturate both kinds of adrenoceptors. There may also be

a large degree of non-specific tissue desensitization occurring that masks any small differential dopamine-specific effect apomorphine may have.

Recently Simon & Van Maanen (1971) reported the existence of dopamine receptors in rat vas deferens. The experimental evidence was based on the use of noradrenaline and dopamine as agonists against phentolamine and apomorphine as antagonists. In the dense, adrenergically-innervated tissues such as rat vas deferens, agonist-antagonist interaction could be markedly influenced by the uptake of noradrenaline by adrenergic neurons. A given blocker could interact with both the adrenergic neurons and the pharmacologic receptors of the effector organ. If apomorphine inhibits the uptake of noradrenaline, the apparent α-adrenoceptor blockade may appear small due to a rise in free concentration of noradrenaline in the synapse. At pharmacologically effective doses dopamine is known to saturate uptake sites (Langer & Trendelenburg, 1969). When the uptake site is saturated, the inhibition may not lead to a rise in free concentration of the drug. The nerve terminal interactions of dopamine and apomorphine may not affect the blockade produced by apomorphine against dopamine at the pharmacologic receptors. Thus, it is not surprising to obtain two apparently different pA_2 values for apomorphine against noradrenaline and dopamine.

If in Fig. 2, Δc and Δd values are compared, it may appear tempting to support the notion that apomorphine-dopamine interaction is specific due to the apparent selectivity in blockade. However, if the data are corrected for the change in sensitivity of the tissue as suggested by Furchgott (1967), the apparent block in selectivity disappears. The values Δc and Δd of Fig. 3 are not significantly different. Since two agonists, phenylephrine or dopamine, are blocked by phentolamine or apomorphine to the same extent, we will have to conclude that dopamine or apomorphine mainly act on the α-adrenoceptor.

Scriabine, Ludden & Morgan (1971) indicated the presence of specific dopamine-like mechanisms in cat heart papillary muscles. Hence, it was of interest to examine the effects of apomorphine and bulbocapnine on guinea-pig atria. Even on β-adrenoceptor bearing atria, apomorphine did not show selective antagonism to dopamine and no distinction could be made between the drug's inhibitory effect on dopamine-induced tachycardia and the histamine-induced response.

Bulbocapnine, a substance reputed to exert effects by a mechanism similar to that of apomorphine also exhibited no selectivity of action when challenged with dopamine or phenylephrine. pA_2 values for bulbocapnine in the presence of either agonist remained the same. It is well accepted that if two agonists act on the same receptor they are expected to be inhibited by a common antagonist to the same degree (Arunlakshana & Schild, 1959) although Furchgott (1967) pointed out the various complexities in evaluating pA_2 or K_B values of antagonists. Hence, phenylephrine, dopamine and bulbocapnine probably act on the same receptor, namely the α- adrenoceptor. Only in one instance did bulbocapnine appear to produce a selectively greater blockade of dopamine (Fig 5, 10^{-5}M). It may be that after α-adrenoceptor blockade by bulbocapnine, more vascular β-adrenoceptors are available for interaction with dopamine. The interaction could result in a greater apparent blockade of dopamine. The results presented in Fig. 6 favour this hypothesis.

The Easson-Stedman theory (1933) predicted that dopamine and (+)-noradrenaline will be equiactive. On tissues such as rat, rabbit, cat and guinea-pig aorta, rat vas deferens and rabbit ileum, spleen and vena cava, the effect of dopamine and

(+)-noradrenaline are, in fact, identical (Patil, 1971; Patil & LaPidus, 1972). Thus, the molecular nature of the interaction for these drugs is presumed to be the same and their receptors indistinguishable. Interestingly, the central neurons of the garden snail have been reported to be selectively inhibited by dopamine. A much higher concentration of (+)-noradrenaline was needed to produce an effect (Woodruff & Walker, 1969). The dissociation of sensitivities indicates the existence of dopamine receptors.

In the peripheral tissues of vertebrates, however, the only evidence that suggests the presence of specific dopamine receptors is provided by Goldberg & others (1968) who showed that dopamine-induced renal vasodilation could be blocked for a short period of time by a limited range of adrenoceptor blocking concentrations. If dopamine receptors are similar to known adrenoceptors, the problem of demonstrating their presence is understandable. We have been unable to obtain any substantial evidence to support the existence of peripheral dopamine receptors. It seems more likely that in the rat vas deferens and rabbit aorta the so-called selective dopamine-like drugs, apomorphine and bulbocapnine, are mainly interacting with α- adrenoceptors.

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