J. Pharm. Pharmacol. 1983, 35: 402–404
Communicated July 27, 1982

Effect of catecholamines on locomotor activity and cyclic AMP in nucleus accumbens in rats

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The addition of dopamine to homogenates of dopamine-rich brain areas such as the nucleus accumbens or striatum enhances the formation of adenosine 3′,5′-cyclic monophosphate (cAMP) (Clement-Cormier et al 1974). Systemic administration of dopamine receptor agonists has been reported to increase levels of cAMP in-vivo in rat limbic system and striatum (Garelis & Neff 1974; Kennedy & Zigmond 1979). However, some investigators have failed to find an increase in rat striatal cAMP content following the administration of dopaminergic drugs (Breese et al 1979; Schmidt et al 1979). The reason for the discrepant reports is not clear as the same species, drugs, and method of killing have been employed in many of these studies.

The present study was conducted to test the effects of dopamine on cAMP-generating systems in-vivo where a parallel behavioural endpoint could be measured. Injection of dopamine into the nucleus accumbens induces a marked locomotor stimulation which is inhibited by neuroleptics but not adrenergic blocking agents (Pijnenburg & van Rossum 1973; Costall et al 1976; Vance & Blumberg 1983). The potency of neuroleptics at antagonizing this hyperactivity roughly parallels their clinical antischizophrenic potency (Costall & Naylor 1976). Thus, it is possible that blockade of the dopamine receptor which mediates locomotor stimulation may be involved in antipsychotic drug actions. The effects of injecting noradrenaline into the nucleus accumbens were also examined as this neurotransmitter has been demonstrated to stimulate adenylate cyclase in-vitro (Blumberg et al 1975, 1976; Horn & Phillipson 1976; Harris 1976) and to increase locomotor activity upon intra-accumbens injection (Costall et al 1976).

Materials and methods

Male Sprague-Dawley rats (175–225 g) under pentobarbitone anaesthesia (50 mg kg^{-1} i.p.) were implanted with 23 gauge stainless steel cannulae bilaterally to the nucleus accumbens (coordinates −6·5 mm ventral, ± 1·4 mm lateral, 3·0 mm anterior to bregma) (Pelligrino & Cushman 1967). Injection cannulae were made from 29 gauge tubing and extended 1 mm below the guide cannulae. Animals were allowed a 6 day recovery period. Pargyline hydrochloride (75 mg kg^{-1} i.p.) was administered 1 h before intra-accumbens injection of 1 μl of 0 9% NaCl (saline) or freshly prepared solutions of dopamine or noradrenaline in saline. After injection rats were placed in Perspex cages (15 × 28 × 36 cm) mounted with a single beam photocell apparatus (Autotron). Locomotor activity was measured as the number of interruptions of the light beam directed across the center of the field. Results are reported in terms of counts per hour in the 30 or 60 min before death.

Rats were killed by 5 s microwave irradiation (General Medical Electronics Model BN K2, 1300W magnetron output) focused through a waveguide onto the animal's head. The nucleus accumbens was dissected on ice and homogenized in 3 ml of 0·3 M perchloric acid containing trace [^{3}H]cAMP for monitoring recovery. After low speed centrifugation the supernatant was poured over Dowex AG 50W-X8 resin (15 × 1 cm column) and the pellet used for protein determination (Lowry et al 1951). cAMP was eluted from the column with 15 ml of 0·01 M HCl (average 93% recovery). The eluate was lyophyllized, reconstituted in 0·1 M acetate buffer pH 4 and assayed for cAMP by the protein binding method of Gilman (1972).

Dopamine hydrochloride, noradrenaline hydrochloride and the chemical reagents for the cAMP and protein assays were purchased from Sigma. The Dowex resin (1 × 2 200–400 mesh) was a BioRad product, [^{3}H]cAMP was purchased from New England Nuclear, and pargyline hydrochloride was supplied by Saber Co.

Results and discussion

Intra-accumbens dopamine caused a profound increase in coordinated locomotor activity which was characterized by a delayed onset. There was no consistent or statistically significant alteration of cAMP content in dopamine-treated rats compared with saline-treated controls. cAMP levels in the nucleus accumbens were increased following direct injection of high concentrations of noradrenaline. A significant increase in cAMP content occurred 30 min after application of 40 or 80 μg noradrenaline. Noradrenaline elicited only a modest increase in motor activity which did not correlate with cAMP changes (Table 1).

In rats, dopamine induces locomotor stimulation after injection into the nucleus accumbens and increases adenylate cyclase activity in homogenates of striatum and nucleus accumbens. The former action is specific-

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Table 1. Effect of intra-accumbens dopamine or noradrenaline on locomotor activity and cAMP concentration.

Drug	Dose (μg)	Time (hour)	Locomotor activity (counts h^{-1}) Saline	Drug	% Change	cAMP (p mol mg^{-1} protein) Saline	Drug	% Change
Dopamine	20	0·5	32 ± 9	57 ± 9	+ 78·1	11·9 ± 1·5	12·3 ± 1·5	+ 3·4
	20	3	49 ± 27	424 ± 96*	+ 765·3	14·6 ± 1·0	15·8 ± 1·1	+ 8·2
	20	5	73 ± 25	958 ± 141*	+1212·3	15·4 ± 1·2	16·4 ± 0·9	+ 6·5
	20	8	58 ± 25	979 ± 227*	+1587·9	14·1 ± 2·4	12·8 ± 1·3	− 9·2
	40	0·5	58 ± 20	140 ± 31	+ 141·4	15·3 ± 0·9	16·3 ± 0·5	+ 6·5
Noradrenaline	20	0·5	47 ± 11	44 ± 8	− 6·4	16·7 ± 1·7	18·6 ± 1·2	+11·4
	20	3	55 ± 14	109 ± 26	+ 98·2	13·5 ± 3·3	13·3 ± 2·2	+ 0·2
	20	5	24 ± 10	150 ± 59*	+ 525·0	14·7 ± 2·6	17·8 ± 3·2	+21·1
	40	0·5	81 ± 18	72 ± 18	− 11·2	12·9 ± 1·0	16·8 ± 1·2*	+30·2
	40	3	51 ± 26	205 ± 60*	+ 302·0	11·6 ± 0·7	15·0 ± 1·5	+29·3
	80	0·5	57 ± 11	38 ± 14	− 33·4	12·9 ± 1·0	17·3 ± 1·6*	+34·1

Results represent the mean ± s.e.m. of at least five rats for each data point.
* $P < 0·05$, Student's t-test.

ally inhibited by neuroleptics in an order of potency which parellels their antischizophrenic action (Costall & Naylor 1976; Vance & Blumberg 1983) while the antagonism of dopamine-sensitive adenylate cyclase by neuroleptics correlates poorly with therapeutic efficacy (Clement-Cormier et al 1974). This discrepancy suggests that dopamine-induced hyperactivity is not mediated by cAMP, a conclusion supported by the inability of intra-accumbens dopamine to increase cAMP in this experiment.

Reports suggesting the dopaminergic hyperactivity is mediated by cAMP find locomotor stimulation following intra-accumbens injection of dibutryl cAMP (Heal et al 1978a) and cholera toxin (Miller & Kelly 1975). In addition, some studies show increased brain concentrations of cAMP following systemic administration of dopaminergic agonists (Garelis & Neff 1974; Heal et al 1978a,b; Kennedy & Zigmond 1979). However, Jackson et al (1975) found no increase in coordinated locomotion after intra-accumbens dibutryl cAMP, and Schmidt et al (1979) and Breese et al (1979) failed to detect increases in brain cAMP levels following systemic administration of dopamine receptor agonists. Stoof & Kebabian (1981) have reported evidence for the existence of two distinct dopamine receptors which modulate striatal adenylate cyclase; one stimulating and the other inhibiting cAMP formation. Such receptors, if functional in-vivo, may be the basis for some of the discrepant findings of the effect of dopaminergic agents on cAMP.

While dopamine is a more potent agonist than noradrenaline in stimulating adenylate cyclase in Tris buffer homogenates of rat striatum or limbic forebrain (Clement-Cormier et al 1974), the converse is true when homogenates or slices of rat brain are prepared in Krebs buffer (Blumberg et al 1976; Horn & Phillipson 1976; Harris 1976). Tris, a non-physiological buffer, has been demonstrated to induce membrane distortions (Chasin et al 1974; Turlapatty et al 1979). Thus, some in-vitro experiments may not reflect in-situ conditions. The level of hyperactivity induced by noradrenaline injection into the nucleus accumbens is less than that induced by dopamine but is prevented by dopamine antagonists not α- or β-adrenoceptor blockers (Costall et al 1976). Noradrenaline has been demonstrated to augment dopamine release in-vitro and in-vitro (Reisine et al 1982). Reserpine pretreatment antagonizes the effects of intra-accumbens noradrenaline (Jackson et al 1975). Thus, the locomotor activity stimulated by noradrenaline may be mediated by dopamine.

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J. Pharm. Pharmacol. 1983, 35: 404–405
Communicated November 24, 1982

Influence of porosity on the contact angle of non-wettable solids

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The wettability of pharmaceutical powders is one of the controlling factors in the dissolution of dosage forms (Zografi & Stamley 1976; Fell & Efentakis 1978; Lerk et al 1978), in the design of suspensions (Parfitt 1973) and in some technological processes (Aulton et al 1977). Wettability is assessed by measurement of contact angles, and the usual methods are the direct method (Harder et al 1970; Ehrhardt 1973; Fell & Efentakis 1979) and the h-ε method (Lerk et al 1976; Fell & Efentakis 1979).

In the direct method a small drop is placed on the surface of the solid compact and its contact angle measured. In the h–ε method the maximum height of a large drop is measured and an equation that allows for the porosity of the compacts used. Thus in comparing contact angles derived by the two methods some discrepancy may arise owing to the fact that in the h–ε method the porosity is taken into account, whereas in the direct method it is neglected. Limited work (Fell & Efentakis 1979) has been done to compare the results obtained by the two different methods.

For surfaces in which the pores cannot be penetrated (contact angles higher than 90°), porosity can be taken into account by applying the following equation (Johnson & Dettre 1969; Adamson 1976):

$$\cos \upsilon_a = f_1 \cos \upsilon_t - f_2 \qquad (1)$$

where υ_a = apparent contact angle; υ_t = true contact angle; f_1 = solid surface fraction of compact; f_2 = void surface fraction of compact.

By assuming that surface fraction can be substituted with volume fraction (porosity), as already suggested by other authors (Lerk et al 1976), we can write equation 1 as:

$$\cos \upsilon_a = (1 - \varepsilon_v) \cos \upsilon_t - \varepsilon_v \qquad (2)$$

where ε_v = compact volume porosity. Equation (2) can be rearranged into:

$$\frac{\cos \upsilon_a}{1 - \varepsilon_v} = \cos \upsilon_t - \frac{\varepsilon_v}{1 - \varepsilon_v} \qquad (3)$$

If equation (3) holds, then a plot of

$$\frac{\cos \upsilon_a}{1 - \varepsilon_v} \text{ versus } \frac{\varepsilon_v}{1 - \varepsilon_v} \text{ should be}$$

linear and the intercept equal to the cosine of the true contact angle. Furthermore, at each compact porosity, the apparent contact angle could be transformed into the true value by the following equation, derived by (3):

$$\cos \upsilon_t = \frac{\cos \upsilon_a + \varepsilon_v}{1 - \varepsilon_v} \qquad (4)$$

Methods

Compacts of magnesium stearate (Farmitalia Carlo Erba, Italy) and Eudragit RL (Rohm Pharma, Germany) were prepared by compressing an appropriate weight of powder in a single 1·128 cm diameter flat punch press (Nassovia, Germany), instrumented with piezoelectric load washers (Kistler, Switzerland).

Solid/water contact angles were measured with a Wettability Tester (Lorentzen-Wettre, Sweden). Small drops of demineralized water were placed on the surface of the sample compacts by means of a microsyringe and, after stabilization, the magnified images of drops were projected onto a screen. The contact angle was derived, via a trigonometric relationship, from the height and length of the base of the drop image. At least 5–10 replicates were carried out.

The porosity of compacts was determined from the apparent tablet density (derived from the tablet weight and dimensions) and the powder density (measured with an air-comparison pycnometer, Beckman, USA).

Results and discussion

The apparent water contact angles measured on magnesium stearate and Eudragit RL compacts prepared at different pressures were plotted according to equation

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