Molecular mechanisms of motor effects of dopamine and cholera toxin in chicks

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It is now well established that dopamine in the striatum plays a fundamental role in the control of the extrapyramidal motor system (Hornykiewicz, 1973). In adult fowls dopamine infused into the palaeostriatum augmentatum (homologous to the mammalian striatum), an area particularly rich in dopamine terminals as documented by histofluorescence (Juorio & Vogt, 1967; Gargiulo & Nisticò, 1975), or infused into the hypothalamus following pretreatment with the MAO inhibitor mebanazine, produces deviation of the head contralateral to the side of infusion and with superimposed side-to-side head-neck movements (Marley & Nistico, 1972). A similar pattern was also evoked after infusing apomorphine into the same brain areas (Nistico, 1976). In young chicks and adult fowls cholera toxin given into the same areas caused dose-dependent increases in motor activity, side-to-side head-neck movements, intense vocalization, tachypnoea and finally circling behaviour and escape responses (Nistico, Stephenson & Preziosi, 1976).

The present experiments were undertaken to investigate the role of adenylate cyclase in the central effects of dopamine; cholera toxin was also used because it stimulates this enzyme in the nucleus accumbens (Miller & Kelly, 1975).

Rhode Island Red chicks (7–8 days old) were killed at the same time (10 a.m.) on the day of each experiment to avoid circadian fluctuation of adenylate cyclase activity. The hypothalami were rapidly removed and assayed for adenylate-cyclase by measuring the conversion of ^{32}P-ATP to cyclic ^{32}P-AMP (Krishna, Weiss & Brodie, 1968).

Each reaction tube contained (mM) ATP 0·3, Mg Cl_2 10, tris-HCl 10 (pH 7·4), KCl 5, theophylline 10, pyruvate kinase (70 μg ml^{-1}), phosphoenolpyruvate 2·6 and ^{32}P-ATP (1·5 $\times$ 10^5 counts min^{-1}). The incubations were started by adding 100 μg of protein from 3 pooled hypothalami homogenates (w/v 1 : 10). At the end of the incubation (2 min) at 37° the reactions were stopped by adding 0·1 ml of a solution containing 5 μmol of ATP, 1·5 μmol of cAMP and 0·1 μCi of ^{3}H-cAMP and boiling for 3 min and ^{32}P-cAMP determined by a liquid scintillation counter as previously described (Mandato, Meldolesi & Macchia, 1975).

A time and dose-dependent formation of 3′,5′-cAMP occurred in chick hypothalamus incubated with dopamine with a maximum at 10^{-4} M; NaF (10^{-2} M) more than doubled adenylate cyclase activity compared with controls (Table 1).

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Table 1. *Effects of dopamine alone (DA) and in combination with antagonists at dopamine receptors and at α- and β-adrenoceptors on cAMP formation in homogenates of chick hypothalami.*

Treatment (M)	pmol cAMP formed mg^{-1} protein in 2 min	
None	526 ± 16·9	(12)
NaF 10^{-2}	1330 ± 35·7*	(6)
DA 10^{-9}	542 ± 21·5	(6)
DA 10^{-8}	583 ± 15·6**	(6)
DA 10^{-7}	644 ± 18·3*	(6)
DA 10^{-6}	703 ± 15·6*	(6)
DA 10^{-5}	768 ± 17·5*	(6)
DA 10^{-4}	800 ± 21·1*	(12)
DA 10^{-3}	719 ± 19·4*	(6)
DA 10^{-4} + Fluphenazine 10^{-4}	553 ± 15·9	(6)
DA 10^{-4} + Phentolamine 10^{-4}	706 ± 22·7*	(6)
DA 10^{-4} + Propranolol 10^{-4}	727 ± 21·1*	(5)

* (P <0·01 in comparison with control values).
** (P <0·05 in comparison with control values).

Adenylate cyclase stimulation evoked by dopamine seemed specific since it was more effectively antagonized by the dopamine receptor antagonist fluphenazine (10^{-4} M) than by antagonists at α- [phentolamine (10^{-4} M)] and β- [propranolol (10^{-4} M)] adrenoceptors (Table 1). Cholera toxin (2·3 μg ml^{-1}) *in vitro* in comparison with control values (530 ± 6·7 pmol cAMP mg^{-1} protein in 2 min) (n = 6) produced a significant (P <0·01) increase in cAMP formation (695 ± 7·9) (n = 8). *In vivo* intrahypothalamic infusion (see Methods in Marley & Nistico, 1972) of cholera toxin, 1·2 μg, significantly (P <0·01) increased cAMP formation (852 ± 11·6 and 845 ± 10·3 respectively) (n = 6) in comparison with control values (609 ± 12·2 pmol cAMP mg^{-1} protein in 2 min). In these experiments the chicks were killed and their hypothalami extracted at the time of the maximum increase in motor activity produced by cholera toxin (i.e. 3 and 10 min after administration).

These experiments indicate that in chicks adenylate cyclase is involved in the central effects of dopamine; stimulation of adenylate cyclase by cholera toxin suggests that the behavioural effects observed after its intracerebral injection are due to dopamine receptor stimulation.

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REFERENCES

GARGIULO, G. & NISTICO, G. (1975). *Acta Med. Vet.*, **21**, 137–167.
HORNYKIEWICZ, O. (1973). *Br. med. Bull.*, **29**, 172–178.
JUORIO, A. V. & VOGT, M. (1967). *J. Physiol. (Lond.)*, **189**, 489–518.
KRISHNA, G. B., WEISS, B. & BRODIE, B. B. (1968). *J. Pharmac. exp. Ther.*, **163**, 379–385.
MANDATO, E., MELDOLESI, M. F. & MACCHIA, V. (1975). *Cancer Res.*, **35**, 3089–3093.
MARLEY, E. & NISTICO, G. (1972). *Br. J. Pharmac.*, **46**, 619–636.
MILLER, R. J. & KELLY, P. H. (1975). *Nature*, **255**, 163–166.
NISTICO, G. (1976). Comm. 1st Joint Meeting Russian and Italian Societies of Pharmacology, Modena, September 1976, p. 141. Modena: Stab. Poligrofico S.T.E.M.
NISTICO, G., STEPHENSON, J. D. & PREZIOSI, P. (1976). *Eur. J. Pharmac.*, **36**, 459–462.

Angiotensin II inhibits the uptake and removal of [^{3}H]metaraminol by rat lung *in vitro*

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Recently we demonstrated that the rat lung tissue is able to take up and concentrate metaraminol by an active transport mechanism (Davila & Davila, 1975a). We also showed that this process may be blocked by various drugs (Davila & Davila, 1975b). It is now well recognized that angiotensin II is able to inhibit uptake of noradrenaline (Palaic & Khairallah, 1967; Peach, Cline & others, 1970) or metaraminol (Davila & Khairallah, 1970; Peach & others, 1970) in several organs. The present studies show *in vitro* evidence for such an inhibitory effect of angiotensin II on the storage and removal of [^{3}H]metaraminol by rat lung.

For this purpose pieces of lung tissue were incubated in Krebs solution according to Davila & Davila (1975a). The uptake of [^{3}H]metaraminol (±)-metaraminol-[7-^{3}H] (N), New England Nuclear Corp., Boston, Mass., USA; specific activity 6·72 Ci mM^{-1}) was measured when it was present in the incubation medium alone or in the presence of angiotensin II (asparagine-1, valine-5 angiotensin II amide, Hypertensin, Ciba-Geigy, Basle, Switzerland). In some experiments the removal of [^{3}H]metaraminol from lung tissue was measured.

Changes in [^{3}H]metaraminol in lung tissue incubated with angiotensin II are presented in Table 1. The octapeptide from 3 to 300 ng ml^{-1} caused a good dose-dependent inhibition of uptake of radioactive metaraminol; the maximal inhibition is about 70%. In a few experiments the rate of removal of [^{3}H]metaraminol from the lung tissue was followed. These experiments were performed with the dose of tested polypeptide which showed about 50% of inhibition of [^{3}H]metaraminol uptake (see Table 1). As shown in Table 2 there is an initial rapid loss of the radioactivity during the first 3 min of the washout period. This is followed by a further slow disappearance of radioactivity. In the presence of angiotensin II during washout the

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Table 1. *The effect of angiotensin II on the uptake of [^{3}H]metaraminol by rat lung.* Equilibration time: 30 min. [^{3}H]Metaraminol: 10 ng ml^{-1}. 5–6 pieces of lung tissue for incubation at 37°. Oxygenation: 95% O_2 and 5% CO_2. Incubation time: 15 min. *P* values: Dunnett's *t*-test in comparison with control.

Dose (ng ml^{-1})	[^{3}H]metaraminol d min^{-1} g^{-1} × 10^3	n*	Inhib. %	*P*
Control				
—	33·23 ± 1·79**	15	0	—
Angiotensin II				
3	32·50 ± 1·29	8	2	>0·05
10	24·20 ± 0·89	10	27	<0·01
30	14·95 ± 1·13	6	55	<0·01
100	8·98 ± 1·71	8	73	<0·01
300	8·96 ± 2·01	5	73	<0·01

* Number of determinations.
** Mean ± s.e.m.

Table 2. *Removal of [^{3}H]metaraminol from rat lung.* Lung tissue was incubated at 37° in the presence of tritiated metaraminol (10 ng ml^{-1}) for 15 min. After incubation lung tissue was washed with fresh metaraminol-free Krebs solution (control) or containing angiotensin II (30 ng ml^{-1}) for 1–20 min. The radioactivity removed from lung tissue was expressed as percentage of the initial radioactivity at zero time (100%). Numbers represent the mean of 6–10 determinations.

% of removed radioactivity						
0	1	3	5 (min)	10	15	20
Control						
0	40	92	99	98	99	99
Angiotensin II						
0	20	45	55	55	56	55