temperature which can be antagonized by substances known to prevent amphetamine hyperthermia favours the classification of the khat alkaloid as a substance with amphetamine-like effects.

I am grateful to Dr O. Braendon (the United Nations Narcotics Laboratory) for a sample of (—)-cathinone hydrochloride, to Cilag AG (Schaffhausen, Switzerland) for pimozide, and to Mrs Judith Noebels for much help with the editing.

April 14, 1980

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Evidence to suggest that dopamine-induced increase in GABA concentrations in chick brain is mediated through cyclic AMP

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We have previously reported (Di Giorgio et al 1979) that, in chicks an oral subacute treatment with L-dopa produces a significant increase in glutamate-decarboxylase (GAD) activity and in the GABA content in the nucleus basalis (the homologue of the mammalian striatum) (Juorio & Vogt 1967; Nisticò & Stephenson 1979).

The present experiments were aimed at substantiating the idea that the functional link between dopaminergic and GABA-ergic mechanisms in the brain (see Pycock et al 1978) has a biochemical basis. Thus the effects of apomorphine, a dopamine receptor agonist, and of (—)-dopa, the precursor of dopamine, given into the IIIrd cerebral ventricle were studied on GABA-ergic mechanisms in the diencephalon.

In addition, we planned to ascertain if the effects of (—)-dopa and apomorphine on GABA-ergic mechanisms are mediated through an increase in 3′,5′-cyclic adenosine monophosphate (cAMP). Therefore, an increase in intracellular cAMP was achieved by giving adenosine, the precursor of cAMP, to chicks pretreated with a phosphodiesterase inhibitor (Mc Ilwain 1971), or by giving the dibutyryl-derivative of cAMP directly as this crosses biological membranes and is more stable to enzymatic degradation (Gessa et al 1970).

Rhode Island Red chicks, 7 days old were kept in a cage maintained at thermoneutral ambient temperature for young chicks (Marley & Stephenson 1970). Cannulae were chronically implanted into the IIIrd cerebral ventricle by means of a 10 μl Hamilton syringe; the infusion rate was 1 μl min^{-1} and the maximum infusate volume was 2 μl. Control infusions of 1–2 μl of the vehicle (pyrogen free dist. H_2O) were without effects on behaviour and body temperature. The diencephalon was quickly dissected out and frozen in liquid nitrogen. GAD activity was assayed by measurement of the $^{14}CO_2$ formed from L-[1-^{14}C]glutamic acid in a liquid scintillator according to Nisticò et al (1979a,b). Preliminary experiments have shown that in our conditions $^{14}CO_2$ loss was approximately 1·5–2%. To avoid non GAD-dependent decarboxylation of glutamate, Triton X-100 (final concn 0·5%) was added to the reaction mixture. GABA content was determined by the enzymatic fluorimetric procedure of Graham & Aprison (1966), as modified by Balcom et al (1975). GABA-transaminase (GABA-T) activity was determined by a radiometric assay (Less & Weiner 1975).

(—)-Dopa and adenosine were from Fluka, apomorphine hydrochloride from Macfarlan Smith Ltd., aminophylline from Macarthys Ltd. Essex, dibutyryl-cAMP from Sigma Chemical Co.), L-[1-^{14}C]glutamic acid and U-[-^{14}C]-γ-aminobutyric acid from Amersham, U.K. GABAase was from Sigma Chemical Co., St. Louis, USA; α-ketoglutaric acid, NADP and NADPH, from Boehringer Mannheim Gmbh, Germany. Other compounds used were the highest purity commercial products available.

Both (—)-dopa and apomorphine given into the IIIrd cerebral ventricle significantly stimulated GABA synthesis. In particular, (—)-dopa given for four consecutive days (0·25 μmol each day, last administration 30 min before) produced, in comparison with control chicks receiving the same volume of the vehicle, a significant increase in GAD-activity and in GABA content in the diencephalon (Table 1). An increase in GAD activity and in GABA was also observed after apomorphine given intraventricularly for four consecutive days (0·5 μmol each day) (Table 1). No changes were observed in GABA-transaminase activity. A single intraventricular injection of dibutyryl-cAMP (0·2 μmol), or of adenosine (0·2 μmol), in chicks pretreated with aminophylline (100 μmol kg^{-1} i.m. 30 min before) increased GAD activity and GABA content in the diencephalon 15 min later (Table 2).

Previous experiments have shown that in rat striatum (Lloyd & Hornykiewicz 1973) and substantia nigra (Kim & Hassler 1975; Lloyd et al 1977) long-term

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Table 1. Effect of an intraventricular treatment in chicks with (—)-dopa or apomorphine on diencephalon GABA content, and on GAD and GABA-T activity (see text for details).

Treatment	GABA content μmol/100 mg protein	GAD activity μmol CO_2/100 mg protein 60 min	GABA-T activity μmol succinic-semi-aldehyde/100mg protein/60 min
Dist. H_2O (n = 16)	4·67 ± 0·26	18·80 ± 0·43	25·89 ± 1·06
L-Dopa (n = 6)	6·49 ± 0·28*	28·13 ± 0·68*	29·9 ± 0·54
Apomorphine (n = 6)	9·02 ± 0·27*	32·71 ± 2·85*	27·05 ± 0·54

Values are expressed as means ± s.e.m. *$P < 0·001$ in comparison with controls.

Table 2. Effect of an intraventricular treatment in chicks with dibutyryl cAMP or adenosine on diencephalon GABA content, and on GAD and GABA-T activity (see text for details).

Treatment	GABA content μmol/100 mg protein	GAD activity μ mol CO_2/100 mg protein/60 min	GABA-T activity μmol succinic semi-aldehyde/100mg protein/60 min
Dist. H_2O	4·54 ± 0·27 (8)	11·69 ± 0·51 (8)	32·34 ± 1·97 (8)
Dibutyryl-cAMP	6·08 ± 0·32* (8)	14·02 ± 0·47* (8)	31·97 ± 1·08 (8)
Adenosine after pretreatment with amino-phylline	6·72 ± 0·27** (8)	14·62 ± 0·38* (8)	33·75 ± 1·15 (8)

Values are expressed as means ± s.e.m. *$P < 0·005$** $P < 0·001$ in comparison with controls. In brackets the number of experiments.

treatment with (—)-dopa stimulates GAD activity; in Parkinsonian patients, where a decrease in GAD activity occurs in the striatum, chronic treatment with (—)-dopa reverses the decrease in GAD activity (Lloyd & Hornykiewicz 1973). Similarly, in chicks, an oral treatment for 8 consecutive days with (—)-dopa produces a significant increase in n. basalis GAD activity and GABA content (Di Giorgio et al 1979). The present experiments confirm that intraventricular treatment with (—)-dopa as well as with apomorphine, a dopamine agonist, produces an increase in GABA synthesis, thus providing evidence that changes in (—)-dopa are mediated through increased dopamine synthesis. Also apomorphine has been found to increase GABA turnover in rats (Pérez de la Mora et al 1975). Several types of DA receptors have been shown to occur in the brain, one of these is linked to an adenylate cyclase activity (Iversen et al 1979; Kebabian & Calne 1979; Spano et al 1979). In chicks apomorphine and dopamine exert their central effects by stimulation of specific receptors (Marley & Nisticò 1972; Nisticò & Stephenson 1979) with consequent activation of a dopamine sensitive adenylate cyclase and intracellular increase in cAMP (Nisticò et al 1978). In conclusion, the present results show that in conditions of increased dopaminergic activity there is an increased synthesis of GABA and suggest that of the multiple DA receptors, those coupled to adenylate cyclase may regulate GAD activity.

Financial support from Italian Council for Research (CNR, Roma contract n. 79.01.940.04) is gratefully acknowledged.

February 1, 1980

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