The lack of effect of high-dose propranolol treatment on striatal DA receptor number and affinity is consistent with the report of Belmaker et al (1979) that high-dose propranolol therapy has no effect on HVA or prolactin concentrations in human c.s.f. These results suggest that possible antischizophrenic effects of propranolol are not mediated by brain dopaminergic mechanisms.

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Tolerance to increases in striatal acetylcholine concentrations after repeated administration of apomorphine dipivaloyl ester

B. SCATTON*, P. WORMS, *Synthelabo-L.E.R.S., Department of Biology, Neurochemistry and Neuropharmacology Units, 31, Avenue Paul-Vaillant Couturier, 92220 Bagneux, France*

Brain dopamine (DA) target cells become less responsive to DA when they have been stimulated in a sustained manner by direct DA receptor agonists. This view is supported by several pieces of evidence: (1) repeated treatment with apomorphine results in an attenuation (tolerance) of the drug-induced hypothermia in mice (Costentin et al 1975); (2) repeated administration of apomorphine dipivaloyl ester (ADPE) for 3–14 days attenuates the stereotyped behaviour in the rat (Worms & Scatton 1977; Scatton & Worms 1978) and the climbing behaviour in the mouse (Scatton et al 1979) seen after acute treatment with ADPE; (3) concomitantly, the decreases in striatal and limbic homovanillic acid (HVA) concentrations induced in both species by an acute ADPE injection are no longer detected following repeated administration of the drug (Scatton & Worms 1978; Scatton et al 1979). Evidence has been provided that nigrostriatal DA neurons terminate (inter alia) on striatal ACh inter-neurons (Hattori et al 1976; Butcher 1977). To provide further evidence in support of the development of a subsensitivity of striatal DA target cells, we have investigated the effect of a repeated treatment with ADPE on acetylcholine (ACh) concentrations in the rat striatum. Stereotyped behaviour was also measured in the same animals and biochemical and behavioural changes were compared.

Male albino rats (COBS, CD strain, Charles River France), 150 ± 3 g were housed at 22 °C and maintained on a 12 h light/dark cycle with free access to food and water. Apomorphine dipivaloyl ester, synthesized from apomorphine hydrochloride (Siegfried, Germany) (base/ester ratio = 0·53), was suspended in water containing 0·1% Tween 80. Stereotyped behaviour was scored in individual cages as described previously (Worms & Scatton 1977). ACh concentrations were measured in one striatum by the method of Guyenet et al (1975) in which the t.l.c. procedure was modified using methylethylketone–acetic acid–water (4:0·75:1) as the migration solvent which provides a better and faster separation of ACh from choline. Since previous studies (Glick et al 1976; Guyenet et al 1977) have shown that many drugs (including DA agonists) affect striatal ACh concentrations similarly after decapitation or microwave irradiation, rats were decapitated.

For statistical analysis of biochemical and behavioural data the two tailed Student's *t*-test and Mann and Whitney's U-test were used, respectively. Correlation coefficients were determined by linear regression analysis using the method of least squares.

A single ADPE injection induced a significant increase in ACh concentrations in the rat striatum

* Correspondence.

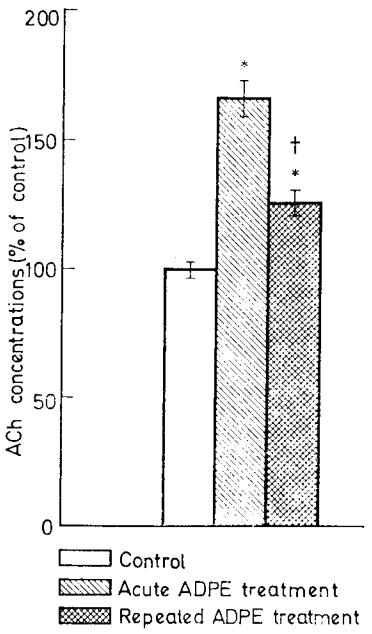

FIG. 1. Striatal ACh concentrations after acute and repeated treatment with ADPE. Rats received single or repeated (twice a day for 14 days) administrations of ADPE (50 mg kg^{-1} i.p.) and were decapitated 2 h after the single or last injection respectively. Results are means with s.e.m. of data obtained from 12 rats (2 experiments) and are expressed as % of control values (ordinate) which were 40 ± 1 nmol g^{-1}. * Versus controls, + versus acute treatment $P < 0.001$.

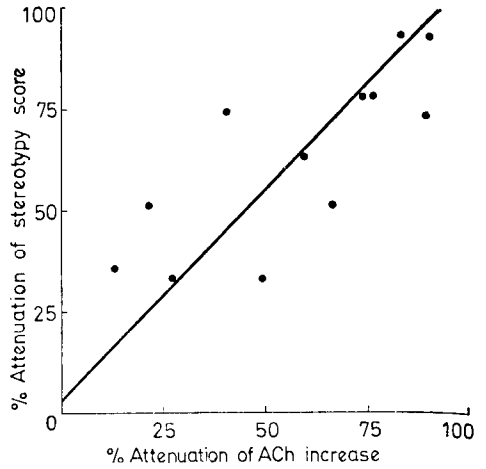

FIG. 2. Repeated treatment with ADPE: correlation betweeen the degrees of tolerance to stereotyped behaviour and to increase in striatal ACh concentrations. Four groups of rats were used. In the first one (control group), rats received i.p. injections of 0·1% Tween 80 twice daily for 2 weeks. The rats of the second group received ADPE (50 mg kg^{-1} i.p.) twice a day for 13 days and were scored for stereotyped behaviour 30 to 180 min after the last ADPE injection. They were then returned to their cages and the treatment with ADPE was continued. On the following day, the animals were decapitated 2 h after the final injection of ADPE for the determination of striatal ACh values. In the third group, rats received 0·1% Tween 80 twice a day for 12 days and a single injection of ADPE (50 mg kg^{-1} i.p.) on the 13th day. Stereotyped behaviour was then scored in parallel with that of the second group. In the fourth group, the animals were treated with 0·1% Tween 80 twice daily for 13 days followed on the 14th day by a single injection of ADPE (50 mg kg^{-1} i.p.) 2 h after which they were decapitated in parallel with the second group for the determination of striatal ACh content.

The degree of tolerance (% attenuation of the acute effect) is defined as the ratio of the difference between the net effects of acute and repeated ADPE treatment to the net effect of acute ADPE treatment. r = 0·81, $P < 0.001$ individual plots. Ordinate: % attenuation of stereotypy score. Abscissa: % attenuation of ACh increase.

compared with controls (Fig. 1). After repeated administration (14 days) of ADPE, striatal ACh values were still increased but to a lesser extent than after acute treatment (Fig. 1). Striatal choline concentrations were not modified either by acute or subacute treatment with ADPE (controls (nmol g^{-1}): 130 ± 4; acute ADPE = 126 ± 6, subacute ADPE = 120 ± 6). Also, as previously reported (Worms & Scatton 1977; Scatton & Worms 1978), repeated administration of ADPE resulted in tolerance to stereotyped behaviour compared with animals receiving a single ADPE injection (mean stereotypy scores cumulated from 0·5 to 3 h after ADPE injection: acute ADPE treatment: 26·7 ± 1·6; subacute ADPE treatment = 11·9 ± 1·7; n = 12, $P < 0.001$). When the degree of tolerance to stereotyped behaviour was plotted against the degree of tolerance to the increase in striatal ACh concentrations for individual animals, a good correlation was obtained (r = 0·81, $P < 0.001$). (Fig. 2).

Several studies have shown than the changes in the concentrations of ACh are in general inversely related to modifications of the transmitter turnover (see Guyenet et al 1975). Thus, the determination of ACh concentration appears to be an index of change in the activity of cholinergic neurons. The present data show that repeated treatment with ADPE results in an attenuation of the increase in striatal ACh concentrations seen after acute treatment with the compound. This effect may be linked to induction of ACh esterase after repeated administration of ADPE. However, this is unlikely as neither apomorphine itself (McGeer et al 1974) nor ADPE (data not shown) change the activity of this enzyme. In addition, acute or repeated treatments with ADPE do not affect striatal choline concentrations. It is more likely that changes in striatal ACh concentration induced by ADPE are related to alterations of ACh turnover as previously suggested for apomorphine itself (Guyenet et al 1975). Thus, dopaminergic neurons appear to exert a tonic inhibitory

influence on target cholinergic interneurons in the striatum (Stadler et al 1973; Lloyd 1978). The increase in striatal ACh concentrations seen after a single ADPE administration may therefore be related to a decrease in ACh turnover subsequent to the stimulation of postsynaptic DA receptors. Tolerance to the increase in striatal ACh concentrations occurring after repeated administration of ADPE may then be related to a decreased responsiveness of DA target cells (cholinergic neurons) during their sustained stimulation by the DA agonist. Apomorphine has been found to increase the turnover rate of 5-HT (Grabowska 1975) and GABA Perez de la Mora et al 1976) in striatum. As some cholinergic neurons in the rat striatum appear to be modulated by 5-HT-ergic (Butcher 1977) and GABA ergic (Javoy et al 1977; Scatton & Bartholini 1979) inputs, it cannot be excluded that the attenuation of the increase in striatal ACh concentrations seen after repeated administration of ADPE may have resulted from compensatory changes occurring in 5-HT-ergic and/or GABA ergic neurons.

Changes of striatal cholinergic activity during repeated ADPE administration may contribute to the modifications of DA turnover and to the alteration of stereotyped behaviour. Thus, it has been suggested that the nigrostriatal DA system is under an excitatory cholinergic influence in the striatum (Bartholini & Stadler 1976, Lloyd 1978). Accordingly, the attenuation of the decrease in striatal HVA concentrations previously observed after repeated ADPE treatment (Worms & Scatton 1977; Scatton & Worms 1978) may be connected with the normalization of cholinergic neuron activity subsequent to the development of DA target cell hyposensitivity. Moreover, evidence has been provided that the striatal cholinergic sytem is involved in the stereotyped behaviour induced by DA-mimetic agents (for review see Lloyd 1978). For instance, apomorphine-induced stereotypies are potentiated by anti-acetylcholine agents and antagonized by cholinomimetics. In addition, anti-acetylcholine drugs themselves can give rise to stereotypies similar in appearance to those caused by DA mimetics. A decreased responsiveness of cholinergic cells may thus also account, at least partly, for the diminution of stereotyped behaviour observed after repeated ADPE treatment. The correlation between the degrees of tolerance to increase in striatal ACh concentrations and to stereotyped behaviour and the slope (about 1) of the regression line (Fig. 2) support this hypothesis.

The mechanism by which striatal DA target cells decrease their responsiveness during a sustained stimulation of DA neurotransmission by ADPE is possibly connected with a decrease in the number of DA receptors. Thus, repeated treatment with ADPE results in a decrease in the number of high affinity [^{3}H]spiroperidol binding sites in the rat striatum (Scatton et al 1979). This is in agreement with other data indicating a similar decrease in the number of [^{3}H]haloperidol and [^{3}H]spiroperidol binding sites after repeated treatment with direct or indirect DA receptor agonists such as bromocriptine, piribedil or L-dopa (Mishra et al 1978). Moreover, repeated daily administration of bromocriptine leads to a reduction of the stimulant action of DA on cyclic AMP formation in striatal slices (Mishra et al 1978).

In conclusion, sustained stimulation of DA receptors by ADPE results in hyposensitivity of striatal cholinergic cells. The changes in activity of the cholinergic neurons are possibly connected with the modifications of the behavioural action of ADPE.

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