Chronic high dose propranolol does not increase dopamine receptor number

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Although propranolol is usually considered to be a blocker of β-adrenoceptors, some biochemical (Fuxe et al 1976; Weinstock et al 1977; Wiesel 1976) and behavioural (Costall et al 1978) reports suggest that high-dose propranolol modifies brain dopaminergic function. High-dose propranolol has been reported to raise serum prolactin (Nasrallah et al 1977) in the manner of dopamine (DA)-receptor blockers, although others disagree (Hanssen et al 1978). The possibility that high dose propranolol blocks DA receptors would suggest a molecular basis for its reported antischizophrenic action (Yorkston et al 1974) since the clinical efficacy of neuroleptic drugs is correlated with their ability to achieve DA receptor blockade (Snyder 1976; Carlsson 1978). Chronic treatment with neuroleptic drugs increases the number of DA receptors as measured by radioligand binding to brain homogenates (Burt et al 1977). We therefore measured changes in striatal DA receptor number and affinity after chronic treatment with high-dose (±)-propranolol in order to investigate whether it has effects similar to those of neuroleptic DA receptor blockers. Chronic lithium was studied as a contrast treatment because of reports that it affects β-adrenergic transmission (Ebstein et al 1976). Chronic propranolol plus haloperidol was studied because of reports of positive synergism of these two treatments (Yorkston et al 1977).

Rat food containing 0·1% (±)-propranolol, or 0·1% LiCl, or 0·1% propranolol plus 0·01% haloperidol, was prepared by grinding regular rat pellets to a fine powder and thoroughly mixing with drug. Control rats received the same powdered food without drug. Male Sabra strain rats, 125 g, were used and weight gain on this diet was normal. The approximate daily oral dose was 30 mg of propranolol or lithium per rat or 3 mg haloperidol. Rats were killed 4 days after cessation of drug feeding and the striatum was dissected and stored at −70 °C until assayed. The binding of [^{3}H]spiroperidol to striatal homogenate was determined as described by Burt et al (1976). The striatum was homogenized using a glass-Teflon homogenizer in 100 volumes of 50 mM Tris buffer pH 7·7 containing the following components (mM): NaCl 120, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, 0·1% ascorbic acid and 10 μM pargyline. The membranes were collected by centrifugation (50 000 *g* for 10 min) and resuspended in 285 volumes (original wet weight) of buffer. The reaction mixture contained 800 μl membrane suspension, 100 μl [^{3}H]spiroperidol (NEN, 23 Ci mM^{-1} from 0·1 to 1·0 nM (5 different concentrations) and either 100 μl 0·1% ascorbic acid or 10 μM DA (blank) in 0·1% ascorbic acid. After 10 min incubation at 37 °C the reaction was stopped by rapid filtering of the suspension through Whatman GFB glass fibre filters and washing with ice-cold buffer (2 × 10 ml). The filters were counted in 10 cc Instagel after shaking vigorously for 2 h. Specific binding of [^{3}H]spiroperidol was calculated as the number of counts in excess over the DA blank. The number of receptor sites and the K_D was determined by Scatchard plot (Scatchard 1949) for each rat striatum. Plots were fitted wih a standard computer program.

High-dose propranolol did not significantly increase DA receptor number after 3 weeks of in vivo treatment (Table 1). Since some clinical studies used propranolol for longer periods before observing any antischizophrenic effect (Yorkston et al 1977), we treated a second group of animals for 6 weeks with propranolol. There was also no effect of high-dose propranolol on DA-receptor number or affinity constant (Table 1).

Our results show no effect of high-dose (±)-propranolol treatment on the number or affinity of DA receptors in rat striatum after up to 6 weeks of treatment. This contrasts with haloperidol, which in a parallel study was observed to cause a 58·9% increase in DA receptor number after chronic 0·01% haloperidol treatment (Ebstein et al 1979). Propranolol plus haloperidol for three weeks led to a significant increase in receptor number (34·7 ± 1·74, n = 7), which was, however, not significantly different from the increase found with haloperidol alone. Six weeks of chronic lithium, which may, like propranolol, affect β-adrenergic transmission (Ebstein et al 1976; Ebstein & Belmaker in the press), also had no effect on DA receptor number or affinity after 6 weeks of chronic treatment (receptor number = 24·65 ± 0·98, K_D = 0·33 ± 0·08, n = 16), in agreement with the data of Pert et al (1978).

Table 1. The effect of 3 and 6 weeks propranolol treatment on striatal DA receptor number (n) and dissociation constant (K_D) (±s.e.m.).

	Receptor number*	K_D
Control	26·85 ± 1·02 (n = 48)	0·41 ± 0·04 (n = 47)
3 weeks propranolol	29·8 ± 2·51 (n = 8)	0·41 ± 0·14 (n = 8)
6 weeks propranolol	26·39 ± 1·19 (n = 16)	0·29 ± 0·004 (n = 16)

* pmol g^{-1} tissue.

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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

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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

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