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Effect of neuroleptic drugs on central catecholamine turnover assessed using tyrosine- and dopamine-β-hydroxylase inhibitors

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The effects of some neuroleptic drugs on the noradrenaline turnover in the rat brain and spinal cord were studied biochemically and histochemically with the help of a new inhibitor of the enzyme dopamine-β-hydroxylase, bis(4-methyl-1-homopiperazinyl-thiocarbonyl) disulphide; (FLA-63). Haloperidol (1 mg/kg, i.p.), chlorpromazine (5–10 mg/kg, i.p.), pimozide (1–5 mg/kg, i.p.) and fluspirilene (1–5 mg/kg, i.p.) produced an acceleration of the noradrenaline disappearance induced by FLA-63. Clothiapine (1 mg/kg, i.p.) was ineffective. The lowering of brain dopamine after treatment with α-methyltyrosine methylester (H44/68) was markedly enhanced by haloperidol (5 mg/kg, i.p.) and slightly so by chlorpromazine (5 mg/kg, i.p.), whereas both drugs simultaneously caused a significant increase in the noradrenaline loss. Thus, the same results were obtained with dopamine-β-hydroxylase and tyrosine-hydroxylase inhibition on the influence of neuroleptic drugs on the noradrenaline turnover. The acceleration seen after haloperidol and chlorpromazine, but not after pimozide and fluspirilene, could be due to a compensatory activation of noradrenaline neurons produced by a noradrenaline receptor blockade.

Previously, Corrodi, Fuxe & Hökfelt (1967), Andén, Corrodi & others (1967) and Andén, Butcher & others (1970) found that neuroleptics could accelerate the disappearance of central catecholamines induced by the tyrosine hydroxylase inhibitor α-methyltyrosine methylester (H44/68) (Corrodi & Hanson, 1966; Andén, Corrodi & others, 1966). To investigate further the increases in central noradrenaline turnover found after treatment with neuroleptic drugs such as chlorpromazine, clothiapine, haloperidol, pimozide and fluspirilene, and H44/68, similar studies have now been made using the dopamine-β-hydroxylase inhibitor bis(4-methyl-1-homopiperazinylthiocarbonyl) disulphide (FLA-63) (Svensson & Waldeck, 1969; Corrodi & Florvall, 1970; Corrodi, Fuxe & others, 1970a, b). Furthermore, the effects of chlorpromazine and haloperidol on the dopamine turnover have been reinvestigated, using H44/68, since acceleration of the H44/68-induced disappearance of dopamine occurred only after repeated doses of these neuroleptics (Corrodi & others, 1967).

MATERIAL AND METHODS

Male Sprague-Dawley rats, 180–250 g, were used. After drug treatment the temperature was frequently checked and maintained at +36–37° by adjustment of the environmental temperature.

Biochemistry

The effects of the neuroleptic drugs on central noradrenaline turnover were evaluated by studying the decline of the stores in the brain after treatment with supramaximal doses of FLA-63. Chlorpromazine, haloperidol, and clothiapine were given 15 min and pimozide and fluspirilene were given 2 h before FLA-63 (all intraperitoneally). The rats were killed 4 h after FLA-63 treament by rapid decapitation under slight chloroform anaesthesia. Biochemical analysis for noradrenaline was made on whole brain spectrofluorimetrically after cation exchange chromatography and oxidation (Bertler, Carlsson & Rosengren, 1958). In the experiments on dopamine turnover, the rate of decline of the dopamine stores after supramaximal doses of H44/68 was studied. Chlorpromazine and haloperidol were given intraperitoneally 15 min before the H44/68 and the rats were killed 4 h later by rapid decapitation. Brain dopamine concentrations were analysed spectrofluorimetrically after cation exchange chromatography and oxidation (Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962). In these experiments, the noradrenaline concentrations in whole brain and spinal cord were also measured as described above.

Statistical significance was calculated by Student's *t*-test for differences between means or after pairing of samples from the same experiment.

Histochemistry

The animals were treated in the same way as in the biochemical experiments. The decline of the noradrenaline and dopamine stores after FLA-63 and H44/68 was evaluated with the histochemical fluorescence technique for the demonstration of catecholamines and 5-HT (Falck, Hillarp & others, 1962; Hillarp, Fuxe & Dahlström, 1966; Corrodi & Jonsson, 1967). A semiquantitative estimation of fluorescence intensity was made on coded slides independently of the biochemical results. It is known that a change in fluorescence intensity reflects a change in amine concentrations (Olson, Hamberger & others, 1968). The noradrenaline nerve terminals of the cortical areas were evaluated with the smear technique (Olson & Ungerstedt, 1970).

Drugs. Chlorpromazine HCl (Leo, Hälsingborg), clothiapine (Wander, Bern), haloperidol (Leo, Hälsingborg), pimozide (Janssen, Beerse), fluspirilene (Janssen, Beerse), DL-α-methyltyrosine methylester HCl (H44/68) (Hässle, Göteborg), bis-(4-methyl-1-homopiperazinylthiocarbonyl)disulphide (FLA-63) (Astra, Södertälje). The doses refer to the form indicated above. The drugs were dissolved in a few drops of glacial acetic acid or N HCl, when necessary, and the final volume was made up by 5·5% glucose or 0·9% saline solution.

RESULTS

Biochemistry

FLA-63. In the doses used, chlorpromazine (5–10 mg/kg), haloperidol (1 mg/kg), pimozide (1 mg/kg) and fluspirilene (1 mg/kg) caused a significant increase in the degree of FLA-63 induced loss of noradrenaline (Tables 1 and 2). Clothiapine (1 mg/kg), on the other hand, did not affect this change.

H44/68. Haloperidol (5 mg/kg) caused a clearcut increase in the H44/68-induced lowering of dopamine concentration whereas the effect of the same dose of chlorpromazine was less and significant only at the 0·025 level (Table 4). On the other

Table 1. *The effects of haloperidol, chlorpromazine and clothiapine (i.p., 4¼ h before killing) on the disappearance of noradrenaline from the rat brain induced by FLA-63 (i.p. 4 h before killing).*

Treatment	N	Noradrenaline %
No drug treatment	4	100 ± 2·3
Haloperidol (1 mg/kg)	4	106 ± 7·1
FLA-63 (25 mg/kg)	4	30 ± 1·9[1]
Haloperidol + FLA-63	4	15 ± 1·1[2]
No drug treatment	4	100 ± 6·5[3]
Chlorpromazine (5 mg/kg)	8	86 ± 2·5[4]
Chlorpromazine (10 mg/kg)	4	89 ± 1·1[5]
Clothiapine (1 mg/kg)	4	99 ± 1·4
FLA-63 (10 mg/kg)	12	49 ± 1·6[6]
Chlorpromazine (5 mg/kg) + FLA-63	8	37 ± 1·9[7]
Chlorpromazine (10 mg/kg) + FLA-63	4	25 ± 1·6[8]
Clothiapine + FLA-63	4	54 ± 6·1[9]

Noradrenaline in % of no drug treatment values. Means ± s.e. Each experiment involves the pooling of two brains. N = number of experiments.

[1–2]: $P < 0{\cdot}005$. [3–4]: $P < 0{\cdot}005$. [3–5]: NS. [6–7]: $P < 0{\cdot}001$. [6–8]: $P < 0{\cdot}001$. [6–9]: NS.

hand, the H44/68 induced disappearance of noradrenaline was clearly increased both after the haloperidol and chlorpromazine treatment.

The neuroleptics causing an acceleration of the catecholamine turnover often produced a slight lowering of the endogenous catecholamine concentrations (Tables 1 and 2).

Histochemistry

FLA-63. The FLA-63 induced disappearance of fluorescence in the noradrenaline nerve terminals of the cortex cerebri and the hypothalamus was clearly enhanced by chlorpromazine (10 mg/kg), haloperidol (1 mg/kg), pimozide (5 mg/kg) and fluspirilene (5 mg/kg). Clothiapine (1 mg/kg) was ineffective (Table 3).

H44/68. The H44/68 induced disappearance of fluorescence in the dopamine nerve terminals of the neostriatum was clearly increased after treatment with halo-

Table 2. *The effects of pimozide and fluspirilene* (1 mg/kg, *i.p., 6 h before killing*) *on the noradrenaline disappearance in the rat brain and spinal cord induced by FLA-63* (25 mg/kg, *i.p., 4 h before killing*).

Treatment	Noradrenaline, brain %	Noradrenaline, spinal cord %
No drug treatment	100 ± 7·9[1]	100 ± 5·4[6]
Pimozide	91 ± 11·0	84 ± 2·3[7]
Fluspirilene	88 ± 6·6[2]	91 ± 9·7
FLA-63	29 ± 2·3[3]	45 ± 1·7[8]
Pimozide + FLA-63	18 ± 1·0[4]	30 ± 4·6[9]
Fluspirilene + FLA-63	18 ± 0·9[5]	29 ± 2·0[10]

Noradrenaline in % of no drug treatment values. Means ± s.e. (3 experiments). Each experiment involves pooling of the organs from two animals.

[1–2]: NS. [3–4]: $P < 0{\cdot}02$. [3–5]: $P < 0{\cdot}02$. [6–7]: $P < 0{\cdot}05$. [8–9]: $P < 0{\cdot}05$. [8–10]: $P < 0{\cdot}02$.

Table 3. *The effect of neuroleptic drugs on the FLA-63-induced fluorescence disappearance from noradrenaline nerve terminals of the cortex cerebri using the smear technique.*

Treatment	Fluorescence intensity	Effect of FLA-63 induced fluorescence disappearance
No drug treatment	3+ (8)	
FLA-63	2+ (12)	
Chlorpromazine (10 mg/kg) + FLA-63	1+ (5) 1½+ (2)	acceleration
Chlorpromazine (5 mg/kg) + FLA-63	1+ (2) 1½+ (4)	acceleration
Clothiapine (1 mg/kg) + FLA-63	2+ (4)	no effect
Haloperidol (1 mg/kg) + FLA-63	1+ (6)	acceleration
Pimozide (5 mg/kg) + FLA-63	1+ (5) 1½+ (1)	acceleration
Fluspirilene (5 mg/kg) + FLA-63	1+ (4)	acceleration

The neuroleptic drugs were given i.p. 1 h (chlorpromazine, haloperidol, clothiapine) or 2 h (pimozide, fluspirilene) before the FLA-63 (25 mg/kg, i.p.) injection. The rats were killed 4 h after the FLA-63 injection. A semiquantitative estimation of fluorescence intensity was made on coded slides: 3+ = strong intensity, 2+ = moderate intensity, 1+ = weak intensity. When it could not be decided if a fluorescence intensity belonged to one or the other grade, half a plus was added to the lower grade. The value from each animal is the mean of 4–6 estimations. Number of animals in parentheses.

peridol (5 mg/kg; 6 rats) but only slightly after chlorpromazine (10 mg/kg; 6 rats). No significant acceleration was observed in the dopamine terminals of the median eminence. The disappearance of fluorescence from the noradrenaline nerve terminals of the cortex cerebri and the hypothalamus was clearly increased after both haloperidol and chlorpromazine, thus confirming previous results (Corrodi & others, 1967).

DISCUSSION

Previous studies using H44/68 have shown that neuroleptic drugs such as chlorpromazine, clothiapine (high dose), haloperidol, pimozide and fluspirilene can increase the turnover of noradrenaline in both the brain and the spinal cord (Corrodi & others, 1967; Andén & others, 1967; Andén & others, 1970). The present findings

Table 4. *The effect of haloperidol* (5 mg/kg, *i.p.*, 4¼ *h*) *and chlorpromazine* (5 mg/kg, *i.p.*, 4¼ *h*) *on the disappearance of dopamine and noradrenaline in the rat central nervous system after treatment with H44/68* (250 mg/kg, *i.p.*, *4 h*).

	H44/68	Haloperidol + H44/68		Chlorpromazine + H44/68	
	% control	% control	Difference ± s.e. from H44/68	% control	Difference ± s.e. from H44/68
Dopamine, brain	28·5	16·5	12·0 ± 1·70 (P <0·001, n = 6)	23·7	4·7 ± 1·45 (P <0·025, n = 6)
Noradrenaline, brain	45·9	27·3	18·6 ± 2·89 (P <0·005, n = 6)	37·7	8·2 ± 1·04 (P <0·001, n = 6)
Noradrenaline, spinal cord	51·9	31·6	20·4 ± 2·78 (P <0·001, n = 6)	40·8	11·1 ± 2·41 (P <0·01, n = 6)

The values are expressed as percentage of the controls not drug treated (brain dopamine concn: 0·62 μg/g; brain noradrenaline concentration: 0·42 μg/g; spinal cord noradrenaline concentration: 0·34 μg/g). The statistical significance of the differences was calculated by Student's t-test after pairing.

further support this view by demonstrating that similar results are obtained also with the dopamine-β-hydroxylase inhibitor FLA-63. The results with FLA-63 exclude that the effects on noradrenaline turnover found in the H44/68 model are secondary to effects on dopamine neurons as has been proposed to explain discrepancies in results by different methods, e.g., that neuroleptic drugs have not always been found to accelerate the disappearance of [^{3}H]noradrenaline (^{3}H-NA) formed from [^{3}H]tyrosine. The present results with FLA-63 also make it unlikely that the changes in amine depletion are due to a drug-inhibitor interaction resulting in changes in the metabolism and distribution of the inhibitors, since the same results were obtained with H44/68 and FLA-63, which differ widely chemically. Also, supramaximal doses of the synthesis inhibitors were used.

Supporting evidence for an increase in central noradrenaline turnover by neuroleptic drugs has also been found by workers using other techniques such as the accumulation and disappearance of labelled noradrenaline after [^{3}H]tyrosine injections (Gey & Pletscher, 1968; Nybäck, Borzecki & Sedvall, 1968; Nybäck & Sedvall, 1970; Persson, 1970). However, in the [^{3}H]tyrosine experiments there are difficulties in demonstrating effects on both ^{3}H-NA accumulation and disappearance (cf. Persson & Waldeck, 1970a). The present results underline the view (Corrodi & others, 1970a, b; Persson & Waldeck, 1970b) that dopamine-β-hydroxylase inhibitors such as FLA-63 are valuable tools in studies on central noradrenaline turnover.

It is also of interest to compare the present and previous chemical results obtained with chlorpromazine, clothiapine and haloperidol with the ability of these drugs to inhibit the L-dopa- or clonidine-induced increase in flexor hindlimb reflex of spinal rats, thus indicating a noradrenaline receptor blockade (Andén & others, 1970 and unpublished data). On the whole there is a good correlation of increase in noradrenaline turnover and blockade of noradrenaline receptors. The increases in turnover obtained with pimozide and fluspirilene are in agreement with those obtained with H44/68 (Andén & others, 1970). Pimozide also causes an increase in the accumulation of ^{3}H-NA from [^{3}H]tyrosine (Nybäck, Schubert & Sedvall, 1970; Persson, 1970) but not in the disappearance of the ^{3}H-NA formed (Nybäck & others, 1970). However, the functional results in the flexor reflex model suggest no blockade of noradrenaline receptors by pimozide and fluspirilene in doses up to 20 mg/kg (Andén & others, 1970, and unpublished data). It was therefore of special importance to establish that evidence for an increased noradrenaline turnover also could be obtained with FLA-63. Thus, pimozide and fluspirilene induce increases in the turnover which cannot be related to a blockade of the receptors. The reason for the increase in turnover is therefore unknown.

Acute treatment with haloperidol caused a clearcut acceleration of the H44/68-induced amine depletion from the neostriatal dopamine nerve terminals, and chlorpromazine induced a weak increase in the H44/68 induced depletion of dopamine in this region. The present chemical results with haloperidol and chlorpromazine agree better than those of Corrodi & others (1967) with the results in rats after unilateral removal of the corpus striatum (Andén, Dahlström & others, 1966) suggesting blockade of dopamine receptors in doses from 0·05–0·1 mg/kg and from 1–2 mg/kg, respectively (Andén & others, 1970). In agreement with the results of Corrodi & others (1967), acute treatment with haloperidol and chlorpromazine did not produce the clear increase in the dopamine turnover in the median eminence seen after multiple treatment with these drugs. The results with chlorpromazine are supported by those

of Nybäck & Sedvall (1969). Since recently it has also been possible to reveal marked increases in dopamine turnover in the H44/68 model after drugs such as perphenazine, spiroperidol, pimozide and fluspirilene (Andén & others, 1970), it seems that H44/68 can be useful to estimate changes in both dopamine and noradrenaline turnover. For studies on noradrenaline turnover alone, the FLA-63 model may be the better.

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