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Effect of nigral lesion on chlorpromazine-induced acceleration of dopamine synthesis from [^{14}C]tyrosine*

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The nigro-neostriatal dopamine pathway of the rat brain was subjected to a unilateral stereotaxic lesion at the level of the hypothalamic-mesencephalic junction. Fifteen days after the operation endogenous dopamine and[^{14}C]dopamine formed *in vivo* from [^{14}C]tyrosine were reduced to about 15% in the striatum ipsilateral to the lesion. Twenty-four h after the lesion the contents of endogenous and labelled dopamine were about the same in the striata of both sides. Chlorpromazine (15 mg/kg) accelerated several fold the accumulation of [^{14}C]dopamine formed from [^{14}C]tyrosine in the striatum on the intact side. However, in the striatum on the side of the lesion, chlorpromazine did not increase the accumulation of [^{14}C]dopamine. The results indicate that chlorpromazine accelerates dopamine synthesis in the striatum by an indirect mechanism, presumably by activating the nerve impulse flow in the nigro-neostriatal dopamine pathway.

Histochemical and biochemical studies have demonstrated a dopamine neuron system extending from the substantia nigra in the brain stem to the nucleus caudatus and putamen (Andén, Carlsson & others, 1964; Dahlström & Fuxe, 1964; Bertler, Falck & others, 1964). Clinical and experimental findings indicate that the nigro-neostriatal dopamine pathway is involved in extrapyramidal motor control (Ehringer & Hornykiewicz, 1960; Carlsson, 1964; Poirier & Sourkes, 1965). Various independent groups have demonstrated that dopamine synthesis in the striatum is accelerated by chlorpromazine (Andén, Roos & Werdinius, 1964; Bernheimer & Hornykiewicz, 1965; Nybäck & Sedvall, 1969), an effect that has been ascribed to a feedback activation of the dopamine neurons induced by a blockade of dopamine receptors (Carlsson & Lindqvist, 1963).

The present investigation was undertaken to test the hypothesis that the chlorpromazine-induced acceleration of dopamine synthesis involves an activation of the nigro-neostriatal dopamine pathway. Nerve impulse activity in the left nigro-neostriatal dopamine pathway was interrupted by a stereotaxic lesion. The accumulation of [^{14}C]dopamine in the left and right striatum during an intravenous infusion of [^{14}C]tyrosine was taken as an index of endogenous dopamine synthesis (Sedvall, Weise & Kopin, 1968; Nybäck & Sedvall, 1970).

METHODS

Male Sprague-Dawley rats, 160–180 g, were used. Under pentobarbitone-sodium anaesthesia (40 mg/kg, i.p.) a stereotaxic lesion was made in the left lateral hypothalamic-mesencephalic junction, where the rostral part of substantia nigra and fibres

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of the dopamine pathway are located (Zeman & Innes, 1963; König & Klippel, 1963; Hökfelt & Ungerstedt, 1969). The lesion was induced mechanically using a needle that had a semicircular tip to produce a spherical lesion with a diameter of 2 mm. By means of a stereotaxic apparatus the needle was lowered vertically into the position for the lesion, turned 360° and removed. The localization and extension of the lesion was verified microscopically in formaldehyde-fixed cresylviolet-stained brain slices (Nybäck, to be published). Sham lesions were made by lowering the needle to a position immediately above the dopamine pathway. It was not rotated before removing.

Fifteen days (chronic brain lesion) or 24 h (acute brain lesion) after the operation the rats were infused intravenously with [^{14}C]tyrosine (446 mCi/mmol, U.L. NEN Chemicals, 25 μCi/animal) for 20 min. The animals were killed immediately after the infusion and the striata of left and right hemispheres were dissected separately (Nybäck & Sedvall, 1969). Striata from three animals were pooled and homogenized in 0·4N $HClO_4$. After centrifugation, endogenous and radioactive tyrosine and dopamine were isolated from the supernatant fluid using chromatography on columns of alumina (Anton & Sayre, 1962), Dowex 50 WX 4 (Musacchio, Goldstein & others, 1966) and Amberlite CG 120 (Lewander & Jonsson, 1968). Endogenous tyrosine and dopamine were determined spectrophotofluorimetrically according to Wong, O'Flynn & Inouye (1964) and Carlsson & Waldeck (1958) respectively. Labelled tyrosine and dopamine were measured by liquid scintillation spectrometry, the efficiency of counting being 80 $\pm$ 5%. For details in the chemical procedure, see Nybäck & Sedvall (1970).

Animals with acute brain lesions were injected intraperitoneally with isotonic saline or chlorpromazine (Hibernal, Leo) (15 mg/kg) 40 min before the infusion of [^{14}C]tyrosine.

RESULTS

After about 3 h, when the animals had recovered from the anaesthesia, they exhibited a tendency to turn towards the side of the lesion. Apart from this the gross behaviour of the animals was normal.

Sham lesion. Fifteen days after the operation the animals were infused with [^{14}C]tyrosine and the contents of endogenous and labelled tyrosine and dopamine in left and right striatum were determined.

Table 1. *Contents of endogenous and labelled tyrosine* (Ty) *and dopamine* (DA) *in the striatum of unoperated and sham lesioned rats after i.v. infusion of* [^{14}C]*tyrosine.* Figures represent mean values of 4–6 determinations $\pm$ s.e.

	Ty (μg/g)	^{14}C-Ty (counts/min $g^{-1} \times 10^{-4}$)	DA (μg/g)	^{14}C-DA (counts/min g^{-1})
Unoperated rats				
left side	8·8	3·74	4·72	1650
	$\pm$2·1	$\pm$0·28	$\pm$0·20	$\pm$126
right side	9·0	3·71	4·34	1550
	$\pm$1·4	$\pm$0·27	$\pm$0·22	$\pm$135
Sham lesioned rats				
operated side	12·1	3·35	4·09	1350
	$\pm$1·2	$\pm$0·44	$\pm$0·40	$\pm$138
unoperated side	12·1	3·39	4·21	1410
	$\pm$1·0	$\pm$0·29	$\pm$0·40	$\pm$119

No significant differences were found in the levels of endogenous and labelled tyrosine and dopamine between the left and right striatum or between sham lesioned animals and unoperated controls (Table 1).

Chronic brain lesion. [^{14}C]Tyrosine was infused intravenously 15 days after the operation and the contents of endogenous and labelled tyrosine and dopamine in left and right striatum were determined.

The levels of endogenous and labelled tyrosine were about the same in both striata (Table 2). The contents of endogenous and labelled dopamine on the side of the lesion were only 13 and 16% respectively of the contents on the intact side. The specific activity of [^{14}C]dopamine, however, seemed to be higher on the lesion side than on the control side.

Table 2. *Contents of endogenous and labelled tyrosine (Ty) and dopamine (DA) in the striatum of rats with a chronic unilateral brain lesion after i.v. infusion of* [^{14}C] Figures represent mean values of 7 determinations ± s.e.

	Ty (μg/g)	^{14}C-Ty (counts/min g^{-1})	^{14}C-Ty sp. act. (counts/min μg^{-1})	DA (μg/g)	^{14}C-DA (counts/min g^{-1})	^{14}C-DA sp. act. (counts/min μg^{-1})
Control side ..	24 ±1·6	31 000 ±4400	1300 ±180	5·8 ±0·36	1600 ±110	290 ±27
Lesion side	30 ±2·0	33 000 ±4200	1100 ±110	0·74* ±0·16	260* ±54	390† ±57

* Differs from control side ($P < 0{\cdot}001$).
† Differs from control side ($P < 0{\cdot}05$).

Acute brain lesion. Twenty-four h after the operation the animals were injected with saline or chlorpromazine (15 mg/kg). Forty min later, [^{14}C]tyrosine was infused and the accumulation of [^{14}C]tyrosine and [^{14}C]dopamine in left and right striatum was determined.

Table 3. *Contents of endogenous and labelled tyrosine (Ty) and dopamine (DA) in the striatum of rats with an acute unilateral brain lesion after i.v. infusion of* [^{14}C]*tyrosine.* Figures represent mean values of 4–5 determinations ± s.e.

		Ty (μg/g)	^{14}C-Ty (counts/min g^{-1})	^{14}C-Ty sp. act. (counts/min μg^{-1})	DA (μg/g)	^{14}C-DA (counts/min g^{-1})	^{14}C-DA sp. act. (counts/min μg^{-1})
Saline	Control side	23 ±2·9	56 000 ±6000	2200 ±90	2·3 ±0·49	1600 ±440	760 ±140
	Lesion side	21 ±2·0	62 000* ±6500	3000* ±160	3·6 ±0·70	1600 ±340	450 ±34
CPZ	Control side	17 ±0·7	66 000 ±4300	3900† ±270	3·0 ±0·46	6100 ±870	2100† ±200
	Lesion side	21 ±2·1	71 000* ±3800	3600 ±480	3·6 ±0·72	2500‡ ±590	760‡ ±150

* Differs from control side ($P < 0{\cdot}05$).
† Differs from saline group ($P < 0{\cdot}01$).
‡ Differs from control side ($P < 0{\cdot}01$) but not from saline group ($P > 0{\cdot}05$).

The levels of endogenous tyrosine and dopamine were about the same in the striata of control and lesion side of both saline and chlorpromazine-treated animals (Table 3). The amount of [^{14}C]tyrosine was possibly higher on the side of the lesion than on the intact side in both saline and drug treated animals. On the control side the specific activity of [^{14}C]tyrosine was increased by chlorpromazine.

[^{14}C]Dopamine accumulated to about the same extent in both striata in the saline group. After chlorpromazine treatment the accumulation of [^{14}C]dopamine in the striatum of the intact side was markedly increased. However, in striatum on the side of the lesion the accumulation of [^{14}C]dopamine was not significantly increased by the drug. The specific activity of [^{14}C]dopamine was about the same in striata of saline-treated animals and on the lesion side after chlorpromazine treatment. In the striatum on the intact side of chlorpromazine-treated animals, however, the specific activity of [^{14}C]dopamine was markedly increased.

DISCUSSION

We have previously demonstrated that chlorpromazine accelerates the accumulation and disappearance of [^{14}C]dopamine formed from [^{14}C]tyrosine in the striatum of rats (Nybäck & Sedvall, 1969; Nybäck to be published). Other independent studies also indicate that chlorpromazine accelerates synthesis and turnover of brain dopamine. Thus accumulation of dopamine metabolites (Carlsson & Lindqvist, 1963; Andén & others, 1964; Bernheimer & Hornykiewicz, 1965) and disappearance of dopamine after tyrosine hydroxylase inhibition (Corrodi, Fuxe & Hökfelt, 1967; Neff & Costa, 1967) are accelerated by chlorpromazine treatment. Therefore it can be concluded that the increased accumulation of [^{14}C]dopamine in the striatum after chlorpromazine is due to, and can be used as an index of, accelerated dopamine synthesis in this brain region.

In the present study unilateral lesions were made in the lateral hypothalamic-mesencephalic junction where fibres of the nigro-neostriatal dopamine pathway are known to pass (Hökfelt & Ungerstedt, 1969). After the chronic lesion, but not after the sham lesion, there was a marked decrease in the contents of endogenous and labelled dopamine in the striatum ipsilateral to the lesion (Tables 1 and 2). This indicates that most fibres of the dopamine pathway were transected by the lesion with consequent degeneration of nerve terminals in the striatum. Degeneration of peripheral adrenergic nerves has previously been shown to result in a loss of both endogenous noradrenaline and the ability of the tissue to accumulate [^{14}C]noradrenaline formed from [^{14}C]tyrosine (Sedvall & others, 1968). In a recent study on monkeys with nigral lesions, Goldstein, Anagnoste & others (1969), obtained similar results.

The chlorpromazine-induced increase in [^{14}C]dopamine accumulation was almost completely abolished by the acute stereotaxic lesion in the lateral hypothalamic-mesencephalic junction (Table 3). The acute lesion did not significantly alter the ability of the striatum of saline-treated animals to form and accumulate [^{14}C]dopamine suggesting that the dopamine terminals were metabolically intact. The results strongly indicate that the acceleration of dopamine synthesis after chlorpromazine treatment is not due to a direct effect of the drug on dopamine nerve terminals in the striatum but requires intact nerve fibres running through the lateral hypothalamic-mesencephalic junction. Evidence for a similar mechanism regarding the effect of chlorpromazine on noradrenaline neurons in the spinal cord has been presented (Andén, Corrodi & others, 1967).

Although it is possible that the present lesion also affected other neurons which might influence dopamine synthesis in striatum, it seems most likely that the abolition of the chlorpromazine effect is due to transection of fibres of the nigro-neostriatal dopamine pathway. The results support the view that chlorpromazine accelerates dopamine synthesis in the striatum by an indirect mechanism, presumably by activating the nerve impulse flow in the nigro-neostriatal dopamine pathway.

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