Enhancement of Tyrosine Hydroxylation within the Brain by Chlorpromazine

Chlorpromazine and other neuroleptics, e.g. haloperidol, have no marked effect on the endogenous levels of cerebral monoamines[1–4]. However, these drugs cause (a) an enhanced accumulation of normetanephrine and 3-methoxytyramine in the brain after blocking of the monoamine oxidase[3], (b) an increased formation of cerebral catecholamines from tyrosine injected intravenously or subcutaneously[5–8], (c) an accelerated disappearance from the brain of catecholamines labelled by previous injection of ^{14}C-3,4-dihydroxyphenylalanine (dopa) (subcutaneous) or of ^{14}C-tyrosine (intravenous)[7], and (d) an enhancement of endogenous homovanillic acid within the brain[4, 10–13].

These results support the theory that neuroleptics accelerate the turnover of catecholamines[3]. However, no direct evidence has been given up to now that these chlorpromazine effects take place within the brain. They might, for instance, be the result of an increased penetration of tyrosine from the blood into the brain or of a major supply of the brain with dopa formed in extracerebral organs of chlorpromazine-treated animals.

In this paper, the effect of chlorpromazine on the fate of labelled tyrosine injected into the cerebral ventricles of rats is reported. For this purpose, several fractions of labelled catechol derivatives have been separated.

Experimental. Male and female albino rats of Wistar origin, weighing 250–280 g, with a permanent cannula in the right lateral ventricle of the brain, were used for the experiments[14]. 10–15 days after cannulation, some animals received chlorpromazine i.p. (10 mg/kg) and were kept at an environmental temperature of 31–32 °C to prevent hypothermia. Controls remained at room temperature. 2 h after chlorpromazine and 1 h before sacrifice, 2.5 µC L-2-^{14}C-tyrosine (specific activity 175 µC/mg) dissolved in 10 µl Na-K phosphate buffer, pH 7.4, 0.1 *M*, were injected into the ventricle. The brain stem (including basal ganglia) was dissected on ice, homogenized in 0.4 *M* $HClO_4$, and the catechols were absorbed on alumina, thereafter eluted with 0.2 *N* HCl and subsequently separated on Dowex 50 X-4 into 4 fractions, i.e. dihydroxylated-deaminated catecholamine metabolites (effluent), dopa, norepinephrine and dopamine[15, 16]. The following modification was performed: After elution of dopa from the column, norepinephrine and dopamine were separated by differential elution using 0.5 and 2 *N* HCl respectively. Standards of labelled dopa, norepinephrine and dopamine added to the homogenates gave recoveries of 80, 72 and 70% respectively, for which the experimental values were corrected. A practically complete separation of the fractions was obtained. The recovery of the first fraction consisting mainly of dihydroxyphenylacetic acid was not calculated.

Results. (1) In the brain stem of rats administered ^{14}C-tyrosine into the cerebral ventricles, chlorpromazine i.p. does not change the overall radioactivity nor the ^{14}C-dopa concentration.

(2) However, chlorpromazine markedly enhances the increase of ^{14}C-norepinephrine, ^{14}C-dopamine and the fraction of ^{14}C-labelled dihydroxylated-deaminated metabolites. These enhancements are observed irrespective of the values of the radioactive fractions being related to the injected radioactivity or to the overall radioactivity in the brain tissue (Table).

Discussion. According to the present experiments, chlorpromazine markedly enhances the concentration of ^{14}C-catecholamines and ^{14}C-catecholamine metabolites in the brain stem of rats injected ^{14}C-tyrosine into the cerebral ventricles. This action is probably not due to an increased uptake of tyrosine or a decreased elimination of tyrosine and/or catechol compounds in the brain tissue, since the overall cerebral radioactivity is the same in controls as in chlorpromazine-treated rats. It has to be considered that some of the radioactive catechols found in the brain tissue originate from extracerebral ^{14}C-dopa. Thus, part of the ^{14}C-tyrosine injected into the cerebral ventricle possibly penetrates via the blood into the extra-

Effect of chlorpromazine (CPZ) in normothermic rast injected ^{14}C-tyrosine into the cerebral ventricles

Fraction	% of injected radioactivity		
	Controls	CPZ	% change
OA	3.27 ± 0.13	3.42 ± 0.19	105.0 ± 4.9
^{14}C-DOPA	0.045 ± 0.008	0.044 ± 0.004	101.3 ± 13.1
^{14}C-NE	0.057 ± 0.009	0.087 ± 0.021	149.5 ± 12.8[a]
^{14}C-DA	0.189 ± 0.012	0.343 ± 0.027	181.4 ± 7.5[a]
^{14}C-DHDM	0.042 ± 0.003	0.099 ± 0.003	236.3 ± 10.1[a]

Fraction	% of OA		
	Controls	CPZ	% change
^{14}C-DOPA	1.44 ± 0.23	1.29 ± 0.05	93.1 ± 10.9
^{14}C-NE	1.77 ± 0.22	2.53 ± 0.46	141.1 ± 7.9[a]
^{14}C-DA	5.90 ± 0.68	10.1 ± 1.30	171.2 ± 14.5[a]
^{14}C-DHDM	1.28 ± 0.05	2.89 ± 0.10	226.6 ± 16.5[a]

^{14}C-Tyrosine was administered 2 h after 10 mg/kg of CPZ i.p. and 1 h before sacrifice. The results are expressed in percent of the injected radioactivity (radioactivity per gram tissue related to the total radioactivity injected) or as percent of the overall cerebral radioactivity. The values represent means with standard error of 3 duplicate determinations each from a pool of 2 brain stems.

[a] $p < 0.01$; OA = overall radioactivity of brain; DOPA = 3,4-dihydroxyphenylalanine; NE = norepinephrine; DA = dopamine; DHDM = dihydroxylated-deaminated derivatives.

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cerebral organs where transformation into ^{14}C-dopa may occur. Part of this ^{14}C-dopa might be taken up by the brain and serve as precursor for ^{14}C-catecholamines and their metabolites. However, according to previous results[5], the small amounts of radioactivity injected intraventricularly in the present experiments would hardly lead to a measurable rise of ^{14}C-catechols if administered extracerebrally (e.g. subcutaneously).

The missing effect of chlorpromazine on the cerebral content of ^{14}C-dopa might be due to the fact that dopa decarboxylase is a highly active enzyme which does not allow dopa to accumulate. Also a slight activation of dopa decarboxylase by chlorpromazine has recently been reported[17]. Acceleration of dopa decarboxylase, however, is hardly a major cause for the increased formation of ^{14}C-catecholamines seen in the present experiments. Thus, according to previous findings[9], chlorpromazine does not enhance the in vivo transformation of ^{14}C-dopa into cerebral ^{14}C-dopamine. Therefore, the increase of cerebral ^{14}C-catecholamines and metabolites after chlorpromazine is probably the consequence of an enhanced hydroxylation of ^{14}C-tyrosine which is thought to be the limiting step in the biosynthesis of catecholamines.

In rats in which the chlorpromazine-induced hypothermia is not prevented the results are less clear. These animals also show a marked rise of ^{14}C-catecholamines and their metabolites in the brain compared with normothermic controls, but at the same time the overall radioactivity of the brain increases.

In conclusion, the present results with the intraventricular injection of ^{14}C-tyrosine in normothermic rats strongly support the hypothesis that chlorpromazine enhances the hydroxylation of tyrosine within the brain. As previously suggested[3,5–8], this effect may be due to a feedback mechanism in consequence of a primary blockade of catecholaminergic receptors by chlorpromazine. A direct activation of tyrosine hydroxylase is unlikely[18,19].

Zusammenfassung. Im Hirnstamm (inklusive basale Ganglien) von normothermen Ratten verstärkt Chlorpromazin die Bildung von Noradrenalin, Dopamin und Catecholaminmetaboliten aus Tyrosin, welches in die Hirnventrikel eingegeben wurde. Die Gesamtradioaktivität sowie die ^{14}C-Dopa-Konzentration im Gehirn werden durch das Medikament nicht verändert. Diese Befunde sprechen für eine zerebrale Aktivierung der Tyrosinhydroxylierung durch Chlorpromazin.

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A Cytochemical Study of Phaeomelanin Formation in Feather Papillae of New Hampshire Chick Embryos

Phaeomelanins are alkali-soluble pigments which as yet have been found only in hair and feathers[1,2]. Investigation of these compounds has proceeded extremely slowly, and it is only recently that the isolation of a number of phaeomelanins from the feathers of New Hampshire chickens has been reported[3,4]. Chemical studies of the isolated pigments, which contain C, H, N, O and S, have suggested that they derive biogenetically from tyrosine and cysteine and that 5-S-cysteinyldopa[5] is an important intermediate in their biosynthesis[6,7].

In order to substantiate this finding we have studied by autoradiography the incorporation of cysteine-3-C^{14} as well as tyrosine-2-C^{14} in the feather papillae of embryonic New Hampshire chick; up till now only this phaeomelanin has been characterized.

Material and methods. Patches of skin were removed from the backs of 19-day-old embryos and sliced into strips about 1 mm wide. Tissue slices were placed in 2.5 ml of 0.1 M phosphate buffer at pH 6.8 containing 2500 units of Penicillin G and incubated for 24 h at 37 °C in a Dubnoff shaker either with 0.3 µc of DL-cysteine-3-C^{14} (34.7 mc/mM) or with 0.25 µc of DL-tyrosine-2-C^{14} (50.0 mc/mM). As a control, slices from the same specimen were treated identically, but in addition to the substrates, sodium diethyldithiocarbamate (0.01 M), a tyrosinase inhibitor, was present. For a second control, 2 other specimens were incubated respectively with 0.3 µc of glycine-2-C^{14} (21.5 mc/mM) and with 0.3 µc of DL-tryptophane (methylene-C^{14}, 52.0 mc/mM). Following incubation, the tissue slices were removed, washed with Hanck's solution and fixed in Carnoy. The specimens were then imbedded in paraffin, sectioned at 5 µ, and autoradiographs made with Kodak NTB-3 liquid emulsion. After 8 days exposure slices were stained with Mayer's hemalum. In other experiments 0.25 µc of labelled cysteine were injected directly into the yolk sac during the 8th day of development. 8 days after the injection the embryos were killed and autoradiographs made as described above.

Results and discussion. Figure 1a and 1b are autoradiographs of epithelial melanocytes incubated with C^{14}-cysteine and C^{14}-tyrosine respectively. It was found that both labelled compounds are incorporated more into melanocytes than into epithelial cells. Also a qualitative comparison revealed no remarkable difference in the uptake of these labelled compounds by the melanocytes.

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