Tabelle III. Hemmung der Phosphodiesterase aus Rattenherzen durch Theophyllin in Abhängigkeit von 3',5'-AMP. Inkubationsansatz 2,1 ml, Inkubationsdauer 20 min

Theophyllin (M/l)							
3',5'-AMP (M/l)	Kontrolle						
	mM P/g Herz	mM P/g Herz	Hemmung	mM P/g Herz	Hemmung	mM P/g Herz	Hemmung
10^{-4}		10^{-4}	%	5×10^{-4}	%	10^{-3}	%
2,5	$6{,}32 \pm 0{,}38$	$5{,}99 \pm 0{,}31$	5	$4{,}83 \pm 0{,}74$	24	$3{,}97 \pm 0{,}52$	37
1	$3{,}76 \pm 0{,}27$	$2{,}75 \pm 0{,}70$	27	$2{,}16 \pm 0{,}25$	43	$1{,}22 \pm 0{,}29$	68

Die in den Tabellen I–III angegebenen Werte sind Mittelwerte aus je 4 Einzelversuchen. Der Streubereich ist als $\pm$ mittlerer Fehler des Mittelwertes angegeben. Signifikante Änderungen gegenüber den Kontrollen sind mit einem * gekennzeichnet.

dem Intensain sehr nahe verwandte Metabolit die gleiche Wirkung auf die Phosphodiesterase hat wie Intensain selbst. Tabelle II zeigt, dass das Verseifungsprodukt nur eine schwache Hemmwirkung besitzt.

Aus Untersuchungen von BUTCHER und SUTHERLAND[9] ist bekannt, dass Xanthin-Derivate die Phosphodiesterase hemmen. Wir untersuchten daher den Einfluss von Theophyllin auf unsere Enzympräparation, einmal, um unsere Versuchsanordnung zu überprüfen, zum anderen, um einen Vergleich zur Wirkungsstärke des Intensain zu erhalten. Die Ergebnisse mit Theophyllin sind in Tabelle III zusammengestellt. Es ist deutlich zu sehen, dass Theophyllin etwa die gleiche Wirkungsstärke wie Intensain zeigt. Eine etwa 50prozentige Hemmung des Enzyms wird bei einer Konzentration von $5 \times 10^{-4} M$ Theophyllin/l in Gegenwart von $10^{-4} M$ 3',5'-AMP/l erreicht.

Summary. Intensain causes in vitro a significant and apparently competitive inhibition of phosphodiesterase, which transforms the 3',5'-AMP to 5'-AMP. These results explain some intensain effects, especially the period of latency to reach the maximum effect, the inotropic action and its reaction under the influence of β-blocking and β-stimulating agents[10–13].

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Acceleration of Turnover of ^{14}C-Catecholamines in Rat Brain by Chlorpromazine

Neuroleptics, e.g. chlorpromazine (CPZ) and haloperidol, are supposed to enhance the cerebral turnover of catecholamines under normothermic conditions. For this assumption only indirect evidence is available. Thus, CPZ (a) increases the formation of ^{14}C-catechol compounds from ^{14}C-tyrosine[1,2], (b) accelerates the disappearance of norepinephrine and dopamine after inhibition of the catecholamine production[3–5], (c) enhances the increase of normetanephrine as well as of 3-methoxytyramine induced by monoamine oxidase inhibitors[6], and (d) leads to accumulation of endogenous homovanillic acid (HVA)[7–12]. It may, however, be argued that (a) fractionation and measurement of the specific radioactivity of the various catecholamines have not yet been carried out, (b/c) neuroleptics might interfere with both the metabolism and the effect of inhibitors of tyrosine hydroxylase and monoamine oxidase respectively, and (d) CPZ possibly inhibits a transport system for the cerebral outflux of HVA[13,14].

The present experiments have been carried out in order to measure the cerebral turnover of catecholamines in a more direct way. For this purpose, the specific radioactivity of cerebral dopamine and norepinephrine was determined in rats given L-2-^{14}C-tyrosine and L-2-^{14}C-3,4-dihydroxyphenylalanine (dopa) respectively.

Experimental. Groups of 3 female rats of a closed colony of Wistar origin (100–110 g body weight) received CPZ i.p. (10 mg/kg) and were subsequently kept in an environment of 31–32 °C to prevent fluctuations in body temperature. Controls were administered saline and remained at room temperature (22 °C). L-2-^{14}C-Tyrosine (5 mg/kg; 105 μC/mg) and L-2-^{14}C-dopa (5 mg/kg; 125 μC/mg) respectively were injected s.c. Immediately after decapitation, the total brain was homogenized in 0.4 N $HClO_4$; the radioactive metabolites were fractionated on Dowex 50, X-4, and subsequently submitted to paper chromatography similarly to previous experiments[15]. The content of total norepinephrine and dopamine respectively was measured spectrophotofluorimetrically[16,17]. The specific radioactivity of the catecholamines is calculated as follows:

$$\frac{\mu\text{g } ^{14}\text{C-amine/g fresh brain} \times 100}{\mu\text{g total amine/g brain}}.$$

Results. Pretreatment with CPZ increases the specific radioactivity of dopamine in rat brain by 74% ($p < 0.01$)

30 and 60 min *after injection of* ^{14}*C-tyrosine*, whereas the specific radioactivity of norepinephrine shows no significant alteration (Table I).

In the brain of animals *given* ^{14}*C-dopa*, subsequent treatment with CPZ decreases the specific activity of dopamine by 46% after 120 and 180 min ($p < 0.05$). CPZ causes a diminution of the specific activity of norepinephrine by maximally 37% which, however, is only significant after 120 min (Table II). The content in ^{14}C-dopa is not markedly changed by CPZ ($p > 0.05$).

The CPZ-induced alterations in the specific radioactivity of the catecholamines are due to changes in the labelled and not in the endogenous catecholamines. The total content of dopamine and norepinephrine is thus not significantly affected by CPZ ($p > 0.05$).

Discussion. The combined experiments with ^{14}C-tyrosine and with ^{14}C-dopa respectively give more direct evidence that CPZ, under normothermic conditions, accelerates the turnover of cerebral dopamine in rats because of the following findings:

(1) Pretreatment with CPZ increases the specific radioactivity of cerebral dopamine in rats injected with ^{14}C-tyrosine (Table I) indicating an increased dopamine formation.

(2) CPZ enhances the decrease of the specific radioactivity of cerebral dopamine labelled by previous injection of ^{14}C-dopa (Table II), probably by an intensified catabolism of the amine.

CPZ also enhances the decrease in the specific activity of norepinephrine in rats injected with ^{14}C-dopa. This suggests that CPZ accelerates the turnover of norepinephrine, too. In agreement with previous results[4], CPZ seems, however, to affect norepinephrine less than dopamine. Thus, the change in the specific activity of norepinephrine is smaller than that of dopamine in ^{14}C-dopa-injected rats and absent in the experiments with ^{14}C-tyrosine.

CPZ is unlikely to increase the cerebral decarboxylation of dopa as shown by earlier experiments[18,19] as well as by the present observation that CPZ does not markedly change the level of ^{14}C-dopa in the brain of ^{14}C-dopa-injected rats. Therefore, the present results confirm the previous suggestion that CPZ increases the hydroxylation of tyrosine[1,2] which seems to be the rate-limiting step of dopamine formation. The acceleration of catecholamine formation by CPZ is presumably due to a feedback mechanism which might be evoked by an enhanced liberation of stored catecholamines. This liberation possibly represents a compensatory reaction to the primary blockade of catecholaminergic receptors by CPZ.

Addendum to the proof. After submission of the manuscript, NYBÄCK et al.[20] reported — in agreement with the present findings — that CPZ increases the conversion of ^{14}C-tyrosine to ^{14}C-dopamine in rat brain in vivo.

Table I. Pretreatment with chlorpromazine (CPZ) in rats injected s.c. with L-2-^{14}C-tyrosine

Min after L-2-^{14}C-tyrosine	Specific radioactivity		p	Change in %
	Controls	Chlorpromazine		
Dopamine				
30	2.41 ± 0.04	3.63 ± 0.30	< 0.01	+ 50
60	2.71 ± 0.15	4.71 ± 0.31	< 0.01	+ 74
110	2.77 ± 0.10	3.06 ± 0.06	> 0.05	+ 11
Norepinephrine				
30	0.77 ± 0.10	0.57 ± 0.07	> 0.05	− 26
60	1.36 ± 0.04	1.48 ± 0.08	> 0.05	+ 9
110	1.77 ± 0.10	1.50 ± 0.03	> 0.05	− 15

10 mg/kg CPZ were injected i.p. 120 min before decapitation. The results represent means of 2–8 experiments ± S.E. In controls without CPZ the total level of cerebral dopamine and norepinephrine was 0.53 ± 0.03 μg/g and 0.30 ± 0.01 μg/g wet brain respectively; in CPZ-treated rats the dopamine level was 0.61 ± 0.04 μg and that of norepinephrine 0.30 ± 0.01 μg respectively.

Table II. Effect of chlorpromazine in rats previously injected with L-2-^{14}C-dopa

Min after		Specific radioactivity		p	Change in %
L-2-^{14}C-dopa	Chlorpromazine	Controls	Chlorpromazine		
Dopamine					
60	–	5.03 ± 0.60	–	–	–
120	60	2.39 ± 0.40	1.28 ± 0.07	< 0.05	− 46
180	120	0.91 ± 0.08	0.49 ± 0.04	< 0.05	− 46
Norepinephrine					
60	–	10.49 ± 1.19	–	–	–
120	60	8.15 ± 1.15	5.02 ± 0.31	< 0.05	− 37
180	120	3.91 ± 0.59	2.90 ± 0.27	> 0.05	− 26

The results represent means ± S.E. of 3 or 4 experiments.

Zusammenfassung. Vorbehandlung mit 10 mg/kg Chlorpromazin (CPZ) i.p. bewirkt bei Ratten, denen ^{14}C-Tyrosin injiziert wurde, eine Zunahme der spezifischen Radioaktivität von ^{14}C-Dopamin im Gehirn. CPZ beschleunigt bei Tieren, deren zerebrale Katecholamine durch L-2-^{14}C-Dopa markiert wurden, den Abfall der spezifischen Radioaktivität von Dopamin und Noradrenalin. Daraus wird geschlossen, dass CPZ den Umsatz zerebraler Katecholamine erhöht.

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