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EFFECTS OF BENZOCTAMINE (30803-Ba, TACITIN®), A NEW PSYCHOACTIVE DRUG, ON CATECHOLAMINE METABOLISM

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Abstract—Benzoctamine (TACITIN®), 1-methylamino-methyldibenzo[b,e]bicyclo[2.2.2] octadiene, is a new psychoactive agent which possesses tranquillizing properties. Its effects on catecholamine metabolism have been studied. Benzoctamine produced no marked change in the catecholamine concentration of various rat organs after either single or repeated treatment and did not inhibit monoamine oxidase or catechol-*O*-methyltransferase activities in liver and brain. It enhanced markedly the incorporation of [^{3}H]tyrosine into [^{3}H]catecholamines in brain and adrenals. It accelerated also the disappearance rate of intracisternally administered [^{3}H]noradrenaline. [^{3}H]noradrenaline uptake in the rat heart and brain was not inhibited. It is concluded that benzoctamine most probably increases the turnover rate of catecholamines.

30803-Ba (TACITIN®) is a 1-amino-alkyl substituted dibenzobicyclo-octadiene[1] which possesses tranquillizing properties. It inhibits aggressiveness without impairing alertness and depresses the activity of the gamma-fibre system. Even high doses produce no anaesthesia and no cataleptic rigidity.[2] The chemical structure of 30803-Ba is given below (Fig. 1):

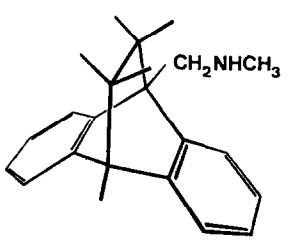

FIG. 1. 1-methylaminomethyl-dibenzo[b,e]bicyclo[2.2.2]octadiene (30803-Ba), benzoctamine, TACITIN®).

Its metabolic fate has been extensively investigated[3,4] and its clinical activity has been described in several publications (e.g. see refs. 5–7). The present paper deals with the study of the influence of 30803-Ba on catecholamine metabolism. Some of the properties of the drug were compared with those of agents known to display psychotropic activities.

METHODS

Male albino rats, weighing 170–230 g, were housed in a controlled illumination environment of 14 hr of light followed by 10 hr of darkness in an animal room with a

constant temperature of 22–23°. They were given free access to unlimited supplies of food and water and were used after an acclimatization of 3–5 weeks.

30803-Ba was administered orally as a suspension in acacia in a volume of 5 ml/kg or subcutaneously in a mixture of physiological saline and polyethylene glycol 400 (3:1) in a volume of 2 ml/kg.

All experiments were carried out on rats except those where the activity on splenic nerve granules was studied.

The rats were sacrificed by decapitation. In some experiments rectal temperature was measured immediately after death.

Protein content was estimated by the method of Lowry *et al.*[8] The statistical methods of Lord[9] and Hogben[10] were used.

Extraction of catecholamines

(a) *Adrenals.* Pairs of adrenals were minced in glass homogenizers, extracted with 5 ml of 10 per cent trichloroacetic acid (TCA) containing 0·01 per cent disodium ethylene diamine tetraacetate (EDTA) and centrifuged. Adrenaline, noradrenaline and dopamine could be estimated fluorimetrically in the supernatants of these extracts without further purification.

(b) *Other tissues.* All other tissues were homogenized twice with 10 per cent TCA using at Polytron (PT 20 OD or PT 10 OD) homogenizer and centrifuged. Catecholamines of the combined supernatants were adsorbed onto acid washed alumina (Woelm, Akt. Stufe I) at pH 8·4, washed with deionized and twice glass distilled water and then eluted twice with 0·25 N HCl. The total volume of the HCl eluate was 3 or 4 ml. All reagents contained 0·01 per cent EDTA. The eluates were centrifuged during 10 min at 30,000 *g* and 0°[11] and stored at −18° until estimation was performed. The recovery of noradrenaline added to heart or brain homogenates averaged 83·4 per cent, that of dopamine added to brain homogenates averaged 80·7 per cent. If not otherwise stated, no corrections for incomplete recovery have been made.

Estimation of catecholamines

The noradrenaline and adrenaline determinations were performed by fluorimetry according to the method of von Euler and Lishajko[12] except that 10 N NaOH was used rather than 5 N for the formation of the lutines. The dopamine content was estimated essentially according to the method of Anton and Sayre.[13] The adrenaline and noradrenaline content of the adrenals was estimated on 40-fold dilutions of the TCA extracts, the dopamine content on the undiluted TCA extracts. Fluorescence of the trihydroxyindoles was measured by duplicate analysis in a Farrand spectrofluorimeter. Known amounts of the catecholamine to be determined were added to an aliquot part of each extract and used as "internal standards". Selective estimations of noradrenaline and dopamine were carried out in the brain, of noradrenaline, dopamine and adrenaline in the adrenals. The catecholamines of the other organs were estimated with noradrenaline as standard.

Estimation of the ^{3}H-noradrenaline uptake and release

(a) *^{3}H-noradrenaline in the heart.* The rats were treated with 30803-Ba or with the reference drugs before or after an intravenous injection of DL[^{3}H]noradrenaline (6·6–10·1 c/mM, New England Nuclear, Boston, Mass., U.S.A., 50 or 100 μc/ml/kg

body wt.). The hearts were removed 1–18 hr later, homogenized and adsorbed onto alumina as described above. Radioactivity was determined in duplicate experiments using 0·2 ml of extract, 2·0 ml ethanol, 1·5 ml methanol and 10·0 ml of a 0·6 per cent butyl-PBD (Scintillator CIBA) solution in toluene. The samples were counted in a Packard-Tri-Carb liquid scintillation spectrometer together with [^{3}H]standards. Under these conditions the counting efficiency was approximately 15 per cent.

In uptake experiments the drugs were given 2 hr before the intravenous injection of [^{3}H]noradrenaline and the retention of [^{3}H]noradrenaline 1 hr later was taken as a measure of its uptake. In release experiments, the drugs were given 30 min after the intravenous injection of [^{3}H]noradrenaline and the hearts were removed 4 or 22 hr later and prepared for [^{3}H]noradrenaline estimation.

(b) *[^{3}H]noradrenaline in the brain.* Essentially the same procedure was used as for the estimation of [^{3}H]noradrenaline in the heart except that the intravenous injection of [^{3}H]noradrenaline was replaced by an intracisternal injection (4 μc/20 μl/rat) according to the method of Schanberg *et al.*[14]

30803-Ba was administered 30 min before or after [^{3}H]noradrenaline. Total radioactivity and [^{3}H]noradrenaline were determined in the whole brain as described above 30 min to 5 hr after [^{3}H]noradrenaline injection.

Estimation of accumulation of [^{3}H]catecholamines in brain and adrenals following intravenous injection of [^{3}H]tyrosine

Rats received 30803-Ba 1 hr before an intravenous injection of 3,5-[H^3]L-tyrosine (1 c/mM, The Radiochemical Centre, Amersham, England) at a dose of 1 mc/ml/kg body wt. The organs were removed 1 hr after [^{3}H]tyrosine injection. The [^{3}H]catechols formed were adsorbed onto alumina as described above and separated by ascending paper chromatography using phenol–0·1 N HCl (85:15 ml) as solvent.

Estimation of endogenous and [^{3}H]tyrosine

The content of endogenous tyrosine was determined fluorometrically by use of the 1-nitroso-2-naphthol condensation method of Waalkes and Udenfriend[15] either in the TCA extracts or after chromatography on Dowex 50 WX4 columns.[16] [^{3}H]tyrosine was assayed only after chromatography on Dowex columns by counting the eluates in the same manner as described above for the eluates from alumina.

Experiments on isolated bovine splenic nerve granules

(a) *Preparation of the granule fraction.* Bovine splenic nerve granules were isolated by differential centrifugation with some modifications of the methods described by von Euler and by Schümann.[17–19] Bovine splenic nerves were obtained from the slaughter house 10–30 min after the animals were killed and were transported to the laboratory packed on ice. All operations for the isolation of the granules were carried out at 0–5°.

The nerves were dissected free from contaminating tissue, weighed and stored overnight at −20°. The frozen nerves were cut into small pieces and homogenized carefully in a 0·3 M sucrose solution using a Polytron PT 20 OD apparatus so that the final homogenate contained 6 ml of sucrose solution per gram of nerve tissue. Coarse particles were removed by centrifugation at 600 *g* for 30 min. The low speed supernatant was centrifuged at 15,000 *g* for 20 min and the new supernatant centrifuged

again at 50,000 *g* for 30 min. The high speed supernatant was discarded and the 50,000 *g* pellets were washed three times with 5 ml ice cooled sucrose solution. Each pellet corresponded to about 2·8 g nerve tissue and contained 2·5–3·3 μg noradrenaline and 11–13 mg protein. The granules were resuspended in 0·3 M sucrose solution in order to determine the influence of drugs on: (a) their ability to take up [^{3}H]noradrenaline and (b) the loss of their endogenous noradrenaline content during incubation.

Estimation of [^{3}H]noradrenaline uptake. The incubation mixture contained: nerve granules corresponding to 0·5 g of nerve tissue, 0·3 M sucrose, 0·009 M sodium phosphate buffer pH 6·8, 0·003 M ATP, 0·003 M $MgCl_2$, 0·1 mM DL[^{3}H]noradrenaline (0·12 μc/μg).

The incubation was carried out at 37° for 20 min together with 0° controls. At the end of the incubation period an aliquot of the suspension was passed through a HAWP 025-Millipore filter and washed twice with ice cold 0·3 M sucrose solution. The filters were dried in counting vials at 90° for 30 min and their radioactivity was estimated by liquid scintillation spectrometry after addition of 10 ml of the scintillator solution mentioned above.

Thin layer chromatography (*n*-butanol saturated with 3 N HCl) of the radioactivity retained in the granules under these conditions showed a main peak of noradrenaline and another small peak which probably corresponds to an acidic metabolite of noradrenaline. For calculation, the total radioactivity found on the Millipore filters was taken as noradrenaline.

During incubation at 37° for 20 min, control granules take up an amount of [^{3}H]noradrenaline corresponding to about 360 per cent (range: 190–554) with respect to the amount found after the same incubation in an ice bath.

(b) *Estimation of the spontaneous noradrenaline release.* The procedure was similar to that used for the estimation of [^{3}H]noradrenaline uptake except that ATP-Mg^{2+} and the tracer dose of [^{3}H]noradrenaline were omitted. After passage through the Millipore filters the noradrenaline remaining in the granules was extracted with 0·4 N perchloric acid and determined fluorimetrically by the trihydroxyindole method mentioned above.

After an incubation at 37° for 20 min control granules lost about 86 per cent (range: 80·2–91·5 per cent) of their noradrenaline content.

Estimation of monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) activities

Rats were treated with 30803-Ba (100 mg/kg, p.o.) once or once daily for 3 days. One hr after the single treatment or 1 hr after the last of three administrations MAO and COMT activities were determined in liver and brain tissues and compared with the enzyme activities of controls treated with vehicle alone.

MAO activity was estimated by radioassay essentially as described by Wurtmann and Axelrod.[20] [^{14}C]tyramine HCl (43·7 mc/mM, The Radiochemical Centre, Amersham, England) was used as substrate at a concentration of 6·25 mμM (10 mμc) for a final incubation mixture of 0·3 ml. The reaction was carried out with 0·250 mg liver and 2 mg brain tissue, respectively. The [^{3}H]deaminated material formed after an injection period of 30 min at 37° was extracted into 6 ml ethylacetate.

For the estimation of COMT activity the method of Axelrod *et al.*[21] was used with some modifications. DL-7-[^{3}H]noradrenaline (6·6 c/mM, New England Nuclear,

TABLE 1. EFFECT OF 30803-Ba ON THE CATECHOLAMINE CONTENT OF RAT ORGANS

Organ	30803-Ba (mg/kg)	Route of administration	Time (hr) between treatment and killing	Noradrenaline (μg/g)				Dopamine (μg/g)			
				n	30803-Ba	*n*	Controls	*n*	30803-Ba	*n*	Controls
Heart	10	p.o.	3	3	0·84 ± 0·075	4	0·81 ± 0·032				
	30	p.o.	3	3	0·79 ± 0·060	4	0·82 ± 0·030				
	100	p.o.	3	6	0·90 ± 0·034*	10	0·82 ± 0·014				
	200	p.o.	3	9	0·94 ± 0·044	9	0·83 ± 0·025				
	20	s.c.	3	7	0·87 ± 0·043	6	0·84 ± 0·048				
	60	s.c.	3	7	0·90 ± 0·031						
	100	p.o.	1	7	0·86 ± 0·020						
			2	5	0·93 ± 0·063	6	0·82 ± 0·035				
			4	11	0·85 ± 0·025						
			20–24	4	0·88 ± 0·037	3	0·82 ± 0·065				
Brain	100	p.o.	1	13	0·405 ± 0·014	11	0·420 ± 0·015	3	0·685 ± 0·063	2	0·736
			2	13	0·362 ± 0·005*	16	0·393 ± 0·005	13	0·735 ± 0·019	16	0·723 ± 0·018
			4	7	0·399 ± 0·012	4	0·416 ± 0·008	7	0·697 ± 0·025	4	0·733 ± 0·043
			6	6	0·406 ± 0·011	6	0·412 ± 0·014	6	0·651 ± 0·022	6	0·662 ± 0·033
			20–24	4	0·412 ± 0·039	5	0·435 ± 0·026				
Spleen	100	p.o.	1	4	0·49 ± 0·044	4	0·69 ± 0·11				
			2	4	0·50 ± 0·090						
			4	4	0·46 ± 0·083						
Salivary glands	100	p.o.	4·5	8	1·55 ± 0·11	7	1·55 ± 0·08				
			18·5	4	1·52 ± 0·08	3	1·49 ± 0·03				
Vas deferens	100	p.o.	3	3	9·73 ± 0·54	6	8·42 ± 0·69				
			4·5	4	8·53 ± 0·32	3	9·32 ± 1·04				
			18·5	4	9·80 ± 0·66	3	8·50 ± 0·62				

Figures represent mean values ± S.E.
n = number of extracts of two organs each.
* = 0·01 < P < 0·05.

Boston, Mass., U.S.A.) was used as substrate and the amount of [^{3}H]normetanephrine formed from [^{3}H]noradrenaline during an incubation period of 20 min at 37° was measured. The incubation mixture contained 15 μmoles phosphate buffer, pH 7·9, 0·5 μmole $MgCl_2$, 5 mμmoles S-adenosyl-methionine, 0·5 mμM (250 mμc) [^{3}H]noradrenaline and 10 mg of liver or 25 mg of brain, respectively, in a final volume of 0·3 ml.

For both MAO and COMT activities, blank values were obtained by carrying out the entire procedure with the corresponding boiled extracts. Radioactivity was determined in duplicate experiments using the butyl-PBD scintillator solution mentioned above. The reactions were linear with time for at least the duration of the incubations.

RESULTS

Effect on endogenous catecholamine stores

The effects of a single administration of various doses of CIBA 30803-Ba on catecholamine content of rat heart, brain, spleen, salivary glands and vas deferens are shown in Table 1. A small and transient increase in the noradrenaline content of the heart was found as well as a small decrease in the noradrenaline but not in the dopamine content of the brain. These effects were of short duration and more constant in the brain than in the heart where they were statistically significant only in some instances. The noradrenaline contents of spleen, salivary glands and vas deferens were unaffected.

In order to avoid the possible influence of the hypothermic effect of 30803-Ba[7] on the catecholamine content, several experiments were carried out on rats kept at an environmental temperature of 30–31° thus preventing the hypothermia produced by

TABLE 2. EFFECT OF 30803-Ba (100 mg/kg, p.o.) ON THE CONTENT OF DOPAMINE IN THE BRAIN AND ON THE CONTENT OF NORADRENALINE IN BRAIN, HEART, SALIVARY GLANDS AND VAS DEFERENS IN RATS KEPT AT AN ENVIRONMENTAL TEMPERATURE OF 30–31°

Organ	Catecholamine measured	Time (hr) between treatment and killing	μg/g			
			n	30803-Ba	*n*	Controls
Brain	Dopamine	0·5	4	0·644 ± 0·029	3	0·689 ± 0·011
		1	4	0·739 ± 0·027		
Brain	Noradrenaline	0·5	4	0·340 ± 0·022	3	0·362 ± 0·009
		1	9	0·342 ± 0·016*	8	0·404 ± 0·015
		2·5	5	0·417 ± 0·024	5	0·434 ± 0·034
Heart	Noradrenaline	0·5	7	0·850 ± 0·059	8	0·830 ± 0·031
		1	7	0·910 ± 0·043		
		2	4	0·970 ± 0·049*	6	0·820 ± 0·035
		4	4	0·880 ± 0·019		
Salivary glands	Noradrenaline	4·5	7	1·500 ± 0·076	6	1·480 ± 0·020
		18·5	6	1·680 ± 0·063*	6	1·420 ± 0·031
Vas deferens	Noradrenaline	4·5	7	7·980 ± 0·240	6	8·960 ± 0·70
		18·5	6	8·450 ± 0·500	6	8·080 ± 0·400

Figures represent mean values ± S.E.
n = number of extracts of two or three organs each.
* = 0·01 < P < 0·05.

TABLE 3. EFFECT OF REPEATED TREATMENT WITH 30803-Ba (100 mg/kg, p.o. DAILY) ON THE CATECHOLAMINE CONTENT OF VARIOUS RAT ORGANS

Duration of treatment		3 days				11 days				11 days			
Hours after the last administration		2				2·5				5·5			
Organ	Catecholamine measured	*n*	30803-Ba	*n*	Controls	*n*	30803-Ba	*n*	Controls	*n*	30803-Ba	*n*	Controls
Heart	NA (μg/g)	7	0·92 ± 0·063	7	0·92 ± 0·063	2	1·12	2	0·81		—		—
Brain	NA (μg/g)	9	0·469 ± 0·018	6	0·415 ± 0·011	8	0·470 ± 0·019	6	0·420 ± 0·007	6	0·461 ± 0·023	4	0·427 ± 0·011
Brain	DA (μg/g)	9	0·674 ± 0·032	6	0·716 ± 0·023	8	0·638 ± 0·011	6	0·690 ± 0·020	6	0·632 ± 0·015	4	0·683 ± 0·029
Salivary Gl.	NA (μg/g)	4	1·62 ± 0·14	4	1·43 ± 0·12	6	1·18 ± 0·077	4	1·59 ± 0·16		—		—
Spleen	NA (μg/g)	4	0·85 ± 0·051	4	0·61 ± 0·21	4	0·79 ± 0·089	3	0·60 ± 0·15		—		—
Adrenals	A (μg/pair)		—		—	6	20·5 ± 0·80	4	18·3 ± 0·44		—		—
	NA (μg/pair)		—		—	6	3·4 ± 0·47	4	4·5 ± 0·52		—		—
	DA (μg/pair)		—		—	6	0·24 ± 0·013	4	0·22 ± 0·014		—		—

Figures represent mean values ± S.E.
n = number of extracts of two organs each.
NA = noradrenaline.
DA = dopamine.
A = adrenaline.

30803-Ba. The results in Table 2 show that essentially the same results were obtained. There was again an indication of a slight noradrenaline decrease in the brain which was also of short duration as at room temperature. On the other hand, a small increase in the noradrenaline content of salivary glands has been observed 18·5 hr after administration of 30803-Ba. Since the little changes produced by a single administration of 30803-Ba were not observed after repeated treatment (Table 3), the biological significance of these small changes is not clear. There was some indication that brain dopamine was lowered and brain noradrenaline increased after repeated treatment with 30803-Ba. However, the amine concentrations found in treated rats were not significantly different ($P > 0{\cdot}05$) from those found in the controls (Table 3).

Effect on the formation of [^{3}H]catechols from [^{3}H]tyrosine

The rate of catecholamine biosynthesis in the brain was examined by injecting rats with radioactive tyrosine and estimating the radioactivity of the catecholamines formed from it in the brain 1 hr after the injection of the labelled precursor. The effect of 30803-Ba was assessed in animals pretreated with the drug one hour before the injection of [^{3}H]tyrosine. Figure 2 shows the effect of 30803-Ba on the

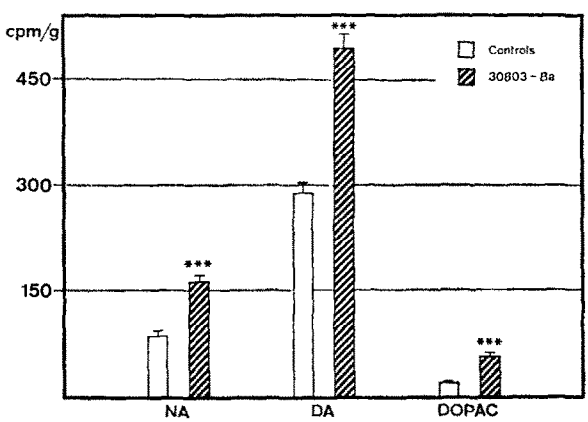

FIG. 2. Effect of 30803-Ba (100 mg/kg, p.o.) on the radioactive catechols found in the rat brain after an intravenous injection of [^{3}H]tyrosine. [^{3}H]tyrosine (1 mc/ml/kg) was administered intravenously 1 hr after 30803-Ba, and 1 hr before killing the animals. The catechols were separated by paper chromatography. Columns represent the mean values ± S.E. of eight extracts of two brains each.

*** $P < 0{\cdot}001$.
NA = noradrenaline.
DA = dopamine.
DOPAC = 3,4-dihydroxyphenylacetic acid.

biosynthesis of noradrenaline and dopamine, respectively. 30803-Ba markedly enhanced the incorporation of radioactivity into noradrenaline as well as into dopamine. In the paper chromatograms used for the separation of individual catecholamines a third radioactive fraction was found, the radioactivity of which was also markedly increased by pretreatment of the animals with 30803-Ba. This peak has been identified as dihydroxyphenylacetic acid (DOPAC).

Essentially the same effect of 30803-Ba was observed when the animals were kept at an environmental temperature of 31° immediately after they had received the drug.

Catecholamine biosynthesis was also examined in the adrenals (Fig. 3). An enhancement of the formation of [^{3}H]catechols from [^{3}H]tyrosine has also been observed although it was less pronounced than in the brain.

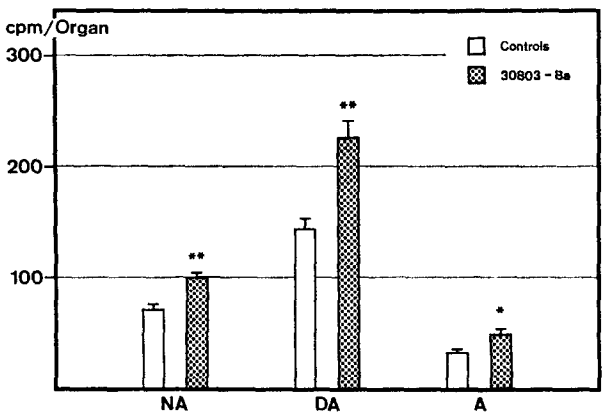

FIG. 3. Effect of 30803-Ba (100 mg/kg, p.o.) on the [^{3}H]catecholamines found in the rat adrenals after an intravenous injection of [^{3}H]tyrosine. Treatment and catecholamine determinations: see legend of Fig. 2. Columns represent the mean values ± S.E. of six extracts of two pairs of adrenals each.

* $0{\cdot}01 < P < 0{\cdot}05$.
** $0{\cdot}001 < P < 0{\cdot}01$.
NA = noradrenaline.
DA = dopamine.
A = adrenaline.

An enhancement of the incorporation of radioactivity from [^{3}H]tyrosine into the total catechols of the rat brain has been reported by Burkard *et al.*[22] under the influence of chlorpromazine. These authors have reported that chlorpromazine decreased the concentration of endogenous tyrosine in the plasma and to a smaller extent in the brain stem of these animals. This effect in itself might result in a greater incorporation of radioactivity into the catecholamines since the same amount of radioactive tracer would be mixed with a lower endogenous tyrosine pool and thus give rise to a higher tyrosine specific activity in the blood.

In order to get a better insight into the mechanism of this enhanced [^{3}H]catecholamine accumulation under the influence of 30803-Ba it was of major importance to determine the concentration of endogenous tyrosine as well as on the tyrosine specific activity. Therefore tyrosine concentrations were determined in plasma and brain, and the tyrosine specific activity was estimated also in plasma and brain 1 hr after the injection of [^{3}H]tyrosine, i.e. at the time when the accumulation of [^{3}H]catecholamines formed from [^{3}H]tyrosine was measured. The effect of 30803-Ba on the concentration of endogenous tyrosine is shown in Table 4. 30803-Ba decreased the plasma levels of tyrosine by about 35 per cent after 1 hr. After 2 and 4 hr the decrease averaged 25 and 20 per cent, respectively. The brain tyrosine levels were not lowered. The tyrosine specific activity in plasma and brain was the same in treated and in control rats (Table 5), because the plasma of treated rats contained not only less endogenous

tyrosine but the amount of radioactive tyrosine was also decreased 1 hr after injection in treated rats in comparison with the controls.

TABLE 4. EFFECT OF 30803-Ba (100 mg/kg, p.o.) ON THE ENDOGENOUS TYROSINE CONTENT IN RAT PLASMA AND BRAIN

Time (hr) between treatment and killing	Concentration of tyrosine					
	Plasma μg/ml			Brain μg/g		
	30803-Ba	Controls	P	30803-Ba	Controls	P
1	10·5 ± 0·5 (15)	16·5 ± 0·7 (12)	< 0·001	18·4 ± 0·9 (15)	16·5 ± 1·1 (12)	> 0·05
1*	12·0 ± 0·2 (6)	16·0 ± 0·2 (5)	< 0·001	23·2 ± 0·7 (3)	19·9 (2)	—
2	12·5 ± 0·3 (12)	16·5 ± 0·7 (11)	< 0·001	15·3 ± 0·9 (15)	16·1 ± 0·9 (13)	> 0·05
4	13·2 ± 1·3 (3)	16·3 (2)	—	18·0 ± 1·1 (6)	16·0 ± 1·1 (4)	> 0.05

Figures represent mean values ± S.E.
The number of extracts is given in parentheses.
Two brains have been pooled for each extract.
* Animals kept at 30–31°.

TABLE 5. EFFECT OF 30803-Ba (100 mg/kg, p.o.) ON THE CONTENTS OF ENDOGENOUS AND RADIOACTIVE TYROSINE IN RAT PLASMA AND BRAIN

Treatment	Plasma			Brain		
	[^{3}H]tyrosine (counts/min × 10^3/ml)	Endogenous tyrosine (μg/ml)	Specific activity (counts/min × 10^3/μg)	[^{3}H]tyrosine (counts/min × 10^3/g)	Endogenous tyrosine (μg/g)	Specific activity (counts/min × 10^3/μg)
(Vehicle)	27·0 ± 1·3	16·3 ± 0·7	1·66 ± 0·04	27·5 ± 2·0	17·3 ± 0·6	1·58 ± 0·07
30803-Ba	19·9 ± 1·0*	12·2 ± 0·3†	1·64 ± 0·08	24·0 ± 1·5 n.s.	15·4 ± 0·7 n.s.	1·55 ± 0·05

[^{3}H]tyrosine was administered intravenously 1 hr after 30803-Ba and 1 hr before killing the animals.
Figures represent the mean values ± S.E. of six extracts of two brains each.
* = 0·001 < P < 0·01.
† = P < 0·001.
n.s = not significant.

Effect on noradrenaline uptake and release mechanisms

(a) *Isolated bovine splenic nerve granules. Effect on [^{3}H]noradrenaline uptake.* 30803-Ba inhibited the uptake of [^{3}H]noradrenaline into bovine splenic nerve granules although rather high concentrations were required (Fig. 4). The threshold concentration which produced an inhibition was found to be approximately 10^{-4} M. Between 10^{-4} M and 10^{-3} M 30803-Ba inhibited the uptake in a concentration dependent manner with an ED_{50} averaging 4×10^{-4} M.

Under the same experimental conditions, chlordiazepoxide also produced a small inhibition of [^{3}H]noradrenaline uptake. It was about 3·5-fold less potent than 30803-Ba. In the other hand, chlorpromazine was 5–10-fold more potent than 30803-Ba, whereas reserpine showed high inhibiting properties in this system with an ED_{50} as small as about 10^{-7} M.

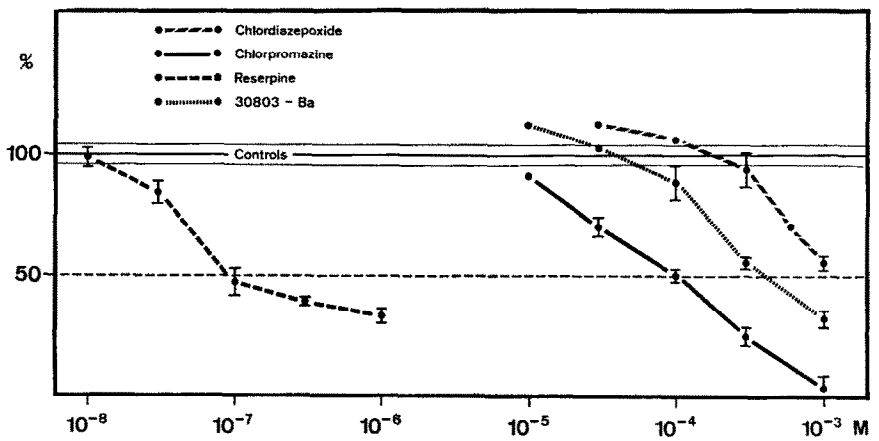

FIG. 4. Effect of 30803-Ba on the uptake of [^{3}H]noradrenaline into isolated bovine splenic nerve granules. Comparison with reserpine, chlorpromazine and chlordiazepoxide. Isolated bovine splenic nerve granules were incubated at 37° for 20 min with 0·1 mM DL[^{3}H]noradrenaline (0·12 $\mu c/\mu g$) in a medium containing 0·003 M ATP-$MgCl_2$. The drugs were not preincubated. Each point represents the mean value ± S.E. of three to six determinations. The points, where no S.E. is given, represent the mean values of two determinations.
Abscissa: molar concentrations of drugs.
Ordinate: percentage inhibition as compared with controls ($n = 10$).

Effect on the spontaneous noradrenaline release. 30803-Ba inhibited slightly the spontaneous release of noradrenaline. The threshold concentration producing this effect was about the same as that causing an inhibition of [^{3}H]noradrenaline uptake. In this case, however, no dose-dependent inhibition could be noted since all concentrations of 30803-Ba between 10^{-4} M and 10^{-3} M inhibited the spontaneous noradrenaline release to about the same extent, i.e. by 15–25 per cent (Fig. 5).

Chlordiazepoxide was inactive up to a concentration of 3×10^{-4} M. Higher concentrations produced a small inhibition which was of the same order of magnitude as that of similar concentration of 30803-Ba.

The effect of chlorpromazine was biphasic. Concentrations ranging from 10^{-5} M to 6×10^{-5} M inhibited the spontaneous release of noradrenaline. Higher concentrations progressively masked this effect as shown from the concentration–response curve which returns gradually to control values. The results given in Fig. 6 indicate that this reversal is probably due to the noradrenaline releasing properties of chlorpromazine itself. In contrast to the active uptake process which is temperature-dependent, this release can even be demonstrated in the cold. It was found that the noradrenaline content of isolated nerve granules was depleted by chlorpromazine already after having been kept during 20 min in an ice bath. This depletion was concentration-dependent between 10^{-4} M and 10^{-3} M (Fig. 6). The other substances assayed did not produce this effect.

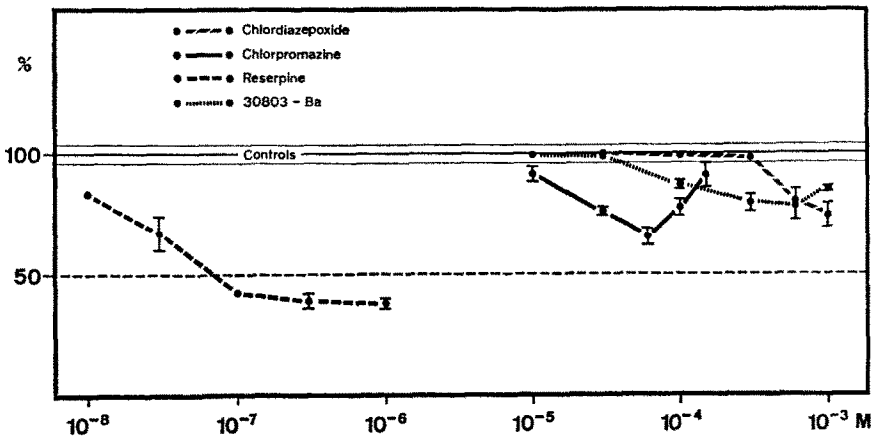

FIG. 5. Effect of 30803-Ba on the spontaneous release of noradrenaline from isolated bovine splenic nerve granules. Comparison with reserpine, chlorpromazine and chlordiazepoxide. Isolated bovine splenic nerve granules were incubated at 37° for 20 min. Their noradrenaline content was compared with that of granules kept in the cold. The drugs were not preincubated. Each point represents the mean value ± S.E. of three to six determinations. The points, where no S.E. is given, represent the mean values of two determinations.

Abscissa: molar concentrations of drugs.

Ordinate: percentage inhibition as compared with controls ($n = 11$).

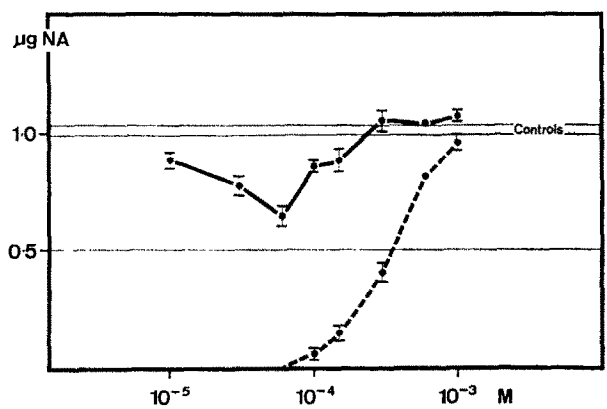

FIG. 6. Effect of chlorpromazine on the spontaneous release of noradrenaline from isolated bovine splenic nerve granules.

Ordinate: μg of noradrenaline lost from isolated granules incubated at 37° for 20 min (solid lines) or kept in an ice bath for 20 min (dotted lines).

Abscissa: molar concentrations of chlorpromazine. Chlorpromazine was not preincubated. Each point represents the mean value ± S.E. of three to six determinations. The points, where no S.E. is given, represent the mean values of two determinations.

(b) *Rat heart. Effect on [^{3}H]noradrenaline uptake.* The effects of various oral or subcutaneous doses of 30803-Ba on the uptake of [^{3}H]noradrenaline in the rat heart are shown in Table 6. They have been compared with those of chlorpromazine and of imipramine. Up to doses of 100 mg/kg p.o. or s.c. 30803-Ba did not inhibit the uptake of [^{3}H]noradrenaline. The amount of total radioactivity and of unchanged [^{3}H]noradrenaline found in the hearts of treated rats 1 hr after the intravenous injection of the

amine was even consistently greater than in the hearts of control rats. After 10 mg/kg s.c. of chlorpromazine, however, there was a significant inhibition of uptake, whereas a dose of 3 mg/kg was ineffective. Under the same experimental conditions, imipramine (6 mg/kg) given orally or subcutaneously inhibited strongly the uptake of [^{3}H]noradrenaline.

TABLE 6. EFFECT OF 30803-Ba, CHLORPROMAZINE AND IMIPRAMINE ON THE UPTAKE OF [^{3}H]NORADRENALINE IN THE HEART

Drug	mg/kg	Orally			Subcutaneously		
		n	[^{3}H]total	[^{3}H]Noradrenaline	*n*	[^{3}H]total	[^{3}H]Noradrenaline
30803-Ba	10	3	134·0 ± 12·0	133·3 ± 14·7	3	126·5 ± 5·4	125·4 ± 10·3
	30	3	122·8 ± 8·5	124·7 ± 10·9	3	134·1 ± 9·6	132·4 ± 11·4
	100	8	107·0 ± 7·2	110·5 ± 10·5	3	124·0 ± 11·6	124·8 ± 14·7
Chlorpromazine	3		—	—	5	104·1 ± 5·8	106·5 ± 5·6
	10		—	—	7	68·2 ± 3·6	67·2 ± 4·7
Imipramine	6	13	38·3 ± 3·5	35·2 ± 4·0	4	15·1 ± 1·1	12·5 ± 1·6

Figures represent mean values ± S.E., in percentage of controls.
n = number of extracts of two hearts each.
[^{3}H]total = total radioactivity.
The mean absolute values ± S.E. of 17 control extracts were:
[^{3}H]total: 113·2 ± 5·3 counts/min . 10^3/g.
[^{3}H]Noradrenaline: 99·3 ± 4·2 counts/min . 10^3/g.

Effect on [^{3}H]noradrenaline release. The effects of the same substances on the release of [^{3}H]noradrenaline from the rat heart were also studied. The rats received first the [^{3}H]noradrenaline and 30 min later the substance to be examined. The amount of radioactive noradrenaline was determined 4 and 22 hr later. The results are shown in Fig. 7.

Between 4 and 22 hr after the administration of 30803-Ba the rate of release of [^{3}H]noradrenaline from the rat heart was not significantly different from that of control animals. Essentially the same pattern of [^{3}H]noradrenaline release was found after chlorpromazine treatment. The effect of imipramine was very different. By contrast to 30803-Ba or to chlorpromazine, imipramine showed a different slope in the disappearance rate of [^{3}H]noradrenaline from the rat heart. The amounts of [^{3}H]noradrenaline found 22 hr after treatment were significantly higher than in controls, indicating a delayed release following the lack of effect or the small acceleration seen during the first hours.

(c) *Rat brain. Effect on [^{3}H]noradrenaline uptake.* The influence of 30803-Ba on the uptake of intracisternally administered [^{3}H]noradrenaline was studied in animals kept at 31° immediately after they had received the drug. [^{3}H]noradrenaline was administered 30 min after 30803-Ba. The total radioactivity and the [^{3}H]noradrenaline were measured in the whole brain 30 min after the [^{3}H]noradrenaline injection. As shown in Table 7, the amount of [^{3}H]noradrenaline taken up by treated rats was the same as that taken up by control rats. The ratios of [^{3}H]noradrenaline to total radioactivity in these extracts are also shown in Table 7. They reflect the relative amount of [^{3}H]noradrenaline to its major [^{3}H]metabolites. In order to gain an exact correlation, the

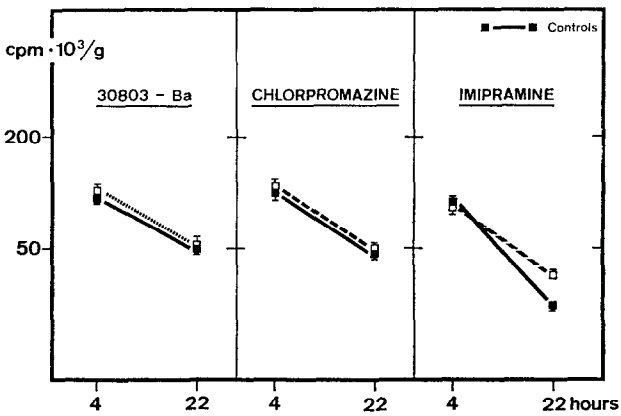

FIG. 7. Effect of 30803-Ba, chlorpromazine and imipramine on the release of [³H]noradrenaline from the rat heart. DL[³H]noradrenaline (100 μc/ml/kg) was injected intravenously 30 min before 30803-Ba (100 mg/kg, p.o.), chlorpromazine (10 mg/kg, s.c.) and imipramine (20 mg/kg, p.o.), respectively. The hearts were removed 4 hr or 22 hr later for [³H]noradrenaline estimation. The symbols represent mean values ± S.E. of nine extracts (30803-Ba), seven extracts (chlorpromazine), and six extracts (imipramine). Six determinations of control release after 4 hr and six after 22 hr were carried out in each group. Two hearts were pooled for each extract.

amounts of [³H]noradrenaline have been corrected in this particular case, with respect to recovery studies carried out with each experiment. The same ratios were found in both groups.

TABLE 7. TOTAL RADIOACTIVITY AND [³H]NORADRENALINE FOUND IN THE RAT BRAIN 30 min AFTER THE INTRACISTERNAL INJECTION OF [³H]NORADRENALINE UNDER THE INFLUENCE OF 30803-Ba (100 mg/kg, p.o.) GIVEN 30 min BEFORE [³H]NORADRENALINE

	Controls	30803-Ba
Total radioactivity (counts/min 10^3/g)	194 ± 19	200 ± 5
[³H]noradrenaline (counts/min 10^3/g)	89 ± 10	91 ± 3
$\frac{[^3H]\text{noradrenaline}^*}{\text{Total radioactivity}}$	0·617 ± 0·007	0·605 ± 0·010
Rectal temperature	37·9 ± 0·12	38·6 ± 0·08

* For this calculation the amounts of [³H]noradrenaline were corrected according to the recovery experiments carried out on the same day (see text).

Figures represent the mean values ± S.E. of six extracts of one brain each.

Effect of [³H]noradrenaline release. In this type of experiment, 30803-Ba was given 30 min after an intracisternal injection of [³H]noradrenaline. As in the latter experiment, the amounts of total radioactivity and of [³H]noradrenaline were measured in the whole brain 30 min, 2 and 5 hr after the [³H]noradrenaline injection. Thus, the

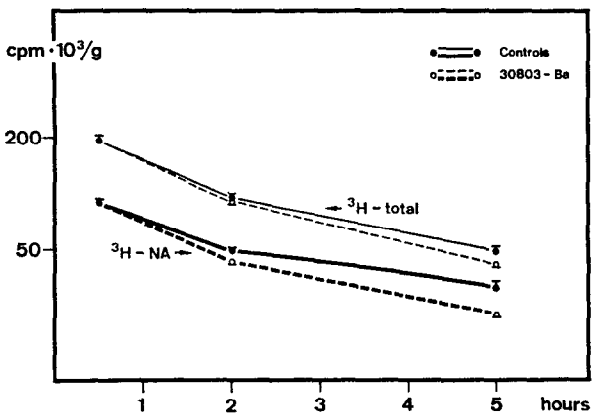

FIG. 8. Effect of 30803-Ba (100 mg/kg, p.o.) on the release of [³H]noradrenaline from the rat brain. DL[³H]noradrenaline (4 μc/20 μl/rat) was injected intracisternally 30 min before 30803-Ba. Total radioactivity ([³H]total) as well as [³H]noradrenaline ([³H]NA) were measured 30 min, 2 hr, and 5 hr after the [³H]noradrenaline injection. Each point represents the mean value ± S.E. of six extracts of one brain each.

30-min values indicate the amounts of radioactivity and of [³H]noradrenaline, respectively, which were present in the brain at the time when 30803-Ba was given. The results of Fig. 8 show that the disappearance rate of the total radioactivity as well as that of the [³H]noradrenaline have been increased by treatment of the animals with 30803-Ba. The ratios of [³H]noradrenaline to total radioactivity in these extracts (Table 8) indicate a greater proportion of metabolites to be present in the brain after 2 hr ($P < 0.05$) and after 5 hr ($P < 0.01$). The increased disappearance rate of [³H]noradrenaline and the higher amount of metabolites found in the drug treated group seems to be the result of a higher turnover of noradrenaline.

Measurements of rectal temperature in these uptake and release experiments show that the hypothermic effect of 30803-Ba was suppressed by keeping the animals at an ambient temperature of 31°.

TABLE 8. RATIO OF [³H]NORADRENALINE TO TOTAL RADIOACTIVITY FOUND IN THE RAT BRAIN AT DIFFERENT TIMES AFTER INTRACISTERNAL INJECTION OF [³H]NORADRENALINE UNDER THE INFLUENCE OF 30803-Ba (100 mg/kg, p.o.) GIVEN AFTER [³H]NORADRENALINE

Time after ³H]noradrenaline injection (hr)	[³H]noradrenaline / Total radioactivity		Rectal temperature	
	Controls	30803-Ba	Controls	30803-Ba
0·5	0·593 ± 0·009	—	37·9 ± 0·20	—
2	0·671 ± 0·014	0·613 ± 0·017*	37·8 ± 0·27	37·5 ± 0·15
5	0·811 ± 0·019	0·695 ± 0·031†	37·9 ± 0·16	37·9 ± 0·13

These values have been calculated from the data presented in Fig. 8.
Figures represent the mean values ± S.E. of six extracts of one brain each.
* $0.01 < P < 0.05$.
† $0.001 < P < 0.01$.

Effect on MAO and COMT activities

Neither MAO activity nor COMT activity in the rat liver and brain were significantly altered by a single administration or by a 3 days treatment with 30803-Ba (Tables 9 and 10).

TABLE 9. EFFECT OF 30803-Ba (100 mg/kg, p.o.) ON MONOAMINE OXIDASE ACTIVITY OF RAT LIVER AND BRAIN

Treatment	Liver			Brain		
	(counts/ min/mg)	μg Protein/mg	Specific activity (counts/ min/μg protein)	(counts/ min/mg)	μg Protein/mg	Specific activity (counts/ min/μg protein)
Vehicle (once)	15989 ± 311	217 ± 4	73·8 ± 2·1	3782 ± 28	160 ± 3	23·7 ± 0·4
30803-Ba (once)	15655 ± 470	218 ± 3	72·0 ± 2·4	3730 ± 77	164 ± 2	22·7 ± 0·5
30803-Ba (three times, once a day)	15842 ± 640	211 ± 3	75·3 ± 3·1	3890 ± 82	163 ± 3	23·9 ± 0·2

The organs were removed 1 hr after the (last) treatment with 30803-Ba.
Figures represent the mean values ± S.E. of five extracts of one organ each.

TABLE 10. EFFECT OF 30803-Ba (100 mg/kg, p.o.) ON CATECHOL-*O*-METHYL-TRANSFERASE ACTIVITY OF RAT LIVER AND BRAIN

Treatment	*n*	Liver			Brain		
		(counts/ min/mg)	μg Protein/mg	Specific activity (counts/ min/μg protein)	(counts/ min/mg)	μg Protein/mg	Specific activity (counts/ min/μg protein)
Vehicle (once)	8	6915 ± 170	218 ± 2	31·7 ± 0·7	229 ± 5	157 ± 3	1·46 ± 0·03
30803-Ba (once)	8	7390 ± 245	214 ± 2	34·7 ± 1·3	256 ± 15	161 ± 2	1·59 ± 0·10
30803-Ba (three times, once a day)	5	7129 ± 138	234 ± 4	30·4 ± 0·5	269 ± 20	167 ± 3	1·61 ± 0·10

The organs were removed 1 hr after the (last) treatment with 30803-Ba.
Figures represent the mean values ± S.E.
n = number of extracts of one organ each.

DISCUSSION

The enhancement of [^{3}H]catecholamine accumulation from [^{3}H]tyrosine in the central nervous system is the most obvious effect on the catecholamine metabolism caused by 30803-Ba. Such an effect has been demonstrated with various neuroleptic drugs principally with chlorpromazine either by measuring the total labelled catechols[22]

or by estimating selectively the amounts of labelled dopamine and noradrenaline found in the brain after administration of radioactive tyrosine.[23,24]

The enhanced accumulation of [^{3}H]catecholamines after injection of [^{3}H]tyrosine may represent an increased activity of tyrosine hydroxylase, the enzyme which is considered as the rate limiting step in the catecholamine biosynthesis.[25]

Like chlorpromazine, 30803-Ba lowered, however, the endogenous content of plasma tyrosine. Therefore the injected [^{3}H]tyrosine would be expected to become less diluted with the endogenous tyrosine pool. Thus, the increase in the labelling of newly formed catechols might be due, at least in part, to the resulting increase in tyrosine specific activity. Since the tyrosine concentration in the brain was not altered and since the specific activities of plasma and brain tyrosine were not different from controls, this effect apparently cannot by itself explain the increase in the hydroxylation of labelled tyrosine in the brain.

The increased catecholamine biosynthesis might well be a consequence of an increased catecholamine turnover. The latter is indicated by the fact that 30803-Ba also accelerated the disappearance of intracisternally injected [^{3}H]noradrenaline. Chlorpromazine was recently shown to cause a marked increase in the [^{14}C]dihydroxylated-deaminated metabolites after intraventricular injection of [^{14}C]tyrosine in the rat brain.[24] This effect was more pronounced than that in the labelling of catecholamines, which indicates that chlorpromazine also increases the catecholamine turnover in the brain.

An increased catecholamine turnover could theoretically be produced by an enhanced activity of catabolic enzymes. Such an effect would not be expected in the present experiments since 30803-Ba did not influence either MAO or COMT activities *in vivo*.

30803-Ba produced an inhibition of [^{3}H]noradrenaline uptake or of noradrenaline release in the isolated nerve granules at relatively high concentrations, i.e. between 3×10^{-5} M and 10^{-4} M. However, after intravenous or intracisternal injection of [^{3}H]noradrenaline no inhibition of [^{3}H]noradrenaline uptake could be detected *in vivo*, even after treatment with a high dose. It seems therefore unlikely that the drug influenced the uptake or release processes at the level of the storage granules in the experiments carried out *in vivo*. This is in agreement with histochemical studies, where the influence of 30803-Ba was examined on the uptake of α-methyl-noradrenaline in the iris, the mesenterial veins, the vas deferens and the eminentia mediana of the reserpinized rat.[26]

The present experiments show that 30803-Ba and chlorpromazine show similarities in their mechanisms of action on catecholamine metabolism but also that both compounds can be clearly differentiated. The principal similarity concerns the action on catecholamine biosynthesis in the central nervous system. Both compounds increase tyrosine hydroxylation in the brain and cause also an enhanced turnover of brain catecholamines.

The most obvious differences between chlorpromazine and 30803-Ba concern the biphasic effect of the first compound on the spontaneous noradrenaline release from isolated nerve granules and its ability to counteract [^{3}H]noradrenaline uptake in the heart. The experiments on isolated granules are consistent with studies on chlorpromazine action reported recently by von Euler and Lishajko[27,28] and may indicate that chlorpromazine can produce some damage at the level of the granula membrane which was not found with 30803-Ba. Chlorpromazine has been found to inhibit the

uptake of [^{3}H]noradrenaline in the rat heart. This effect is in agreement with observations by other authors obtained under different conditions, i.e. inhibition of the uptake of [^{3}H]noradrenaline into several sympathetically innervated organs of the cat,[29] inhibition of the uptake of neurally released noradrenaline also in the cat,[30] inhibition of the uptake of [^{3}H]noradrenaline in the isolated rat heart[31] and in the rat brain *in vivo*.[32] In contrast, 30803-Ba rather enhanced the [^{3}H]noradrenaline uptake in the rat heart and did not influence it in the rat brain *in vivo*.

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