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BLOCKADE OF NORADRENALINE UPTAKE BY 34276-Ba, A NEW ANTIDEPRESSANT DRUG

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Abstract—34276-Ba is 1-(3-methylaminopropyl)-dibenzo[b,e]bicyclo-[2.2.2.] octadiene hydrochloride, an antidepressant drug from a new class of chemical compounds. Its effects on noradrenaline uptake have been studied and compared with those of imipramine or desmethylimipramine. 34276-Ba was found to be a powerful inhibitor of noradrenaline uptake through the nerve cell membrane in several sympathetically innervated organs of the rat, cat and chick *in vivo*. The inhibitory effect was very pronounced in the brain as well as in peripheral tissues. The new antidepressant drug also inhibited the guanethidine-induced depletion of the endogenous noradrenaline stores and the uptake of [^{3}H]metaraminol in the rat heart. Although 34276-Ba caused a concentration dependent inhibition of noradrenaline uptake into isolated bovine splenic nerve granules, it did not alter markedly the endogenous concentration of catecholamines in heart and brain even after repeated daily treatment.

THE MECHANISM principally responsible for rapid inactivation of injected or endogeneously released noradrenaline is its uptake into the adrenergic neurones.[1–3] A number of drugs has been reported to inhibit this inactivation process, among them tricyclic antidepressant drugs, such as imipramine, desmethylimipramine and amitriptyline, thus potentiating the actions of noradrenaline.[4]

34276-Ba (Fig. 1) belongs to a new class of chemical compounds, the amino-alkyl-substituted dibenzo-bicyclo-octadienes[5] and displays antidepressant properties.[6]

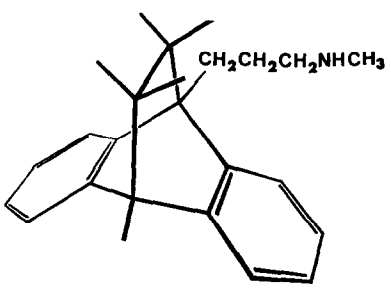

FIG. 1. 34276-Ba: 1-(3-methylaminopropyl)-dibenzo[b,e]bicyclo-[2.2.2] octadiene, hydrochloride.

It seemed of interest to compare the effects of this structurally new antidepressant drug with those of imipramine or desmethylimipramine on noradrenaline uptake mechanisms.

MATERIALS AND METHODS

Uptake of [^3H]noradrenaline

Isolated bovine splenic nerve granules. Splenic nerve granules were isolated by differential centrifugation with some modifications[7] of the methods described by von Euler[8] and by Schümann.[9] [^{3}H]noradrenaline uptake was estimated by incubating suspensions of the granule fractions at 37° for 20 min in a medium containing 0·3 M sucrose, 0·009 M sodium phosphate buffer pH 6·8, 0·003 M ATP, 0·003 M $MgCl_2$ and 0·1 mM DL-[^{3}H]noradrenaline (0·12 μc/μg). The granules were then collected by passage of the suspension through a HAWP 025 Millipore filter, dried, and counted in a Packard-Tri-Carb liquid scintillation spectrometer after addition of 10 ml of a 0·6% butyl PBD (Scintillator CIBA) solution in toluene. Granules suspensions kept in an ice bath during incubation time were used as controls.

Rat: peripheral tissues. Male albino rats (170–230 g body weight) were treated with 34276-Ba or with the reference drugs 2 hr, if not otherwise stated, before an intravenous injection of DL-[^{3}H]noradrenaline (6·6–10·1 c/mM, New England Nuclear, Boston, Mass., U.S.A., 100 μc/ml/kg body weight). 34276-Ba was suspended in acacia gum for oral treatment and dissolved in a mixture of polyethylene glycol 400 and physiological saline (1:4) for subcutaneous or intravenous injections. Control rats received vehicle only. Organs were removed for [^{3}H]noradrenaline estimations 1 hr after the injection of the labelled amine. In one set of experiments they were removed 3, 10, or 30 min after the injection of the labelled amine. The retention of [^{3}H]-noradrenaline at removal time was taken as a measure of its uptake. The organs were then homogenized in 10% trichloroacetic acid using a Polytron (PT 20 OD or PT 10 OD) homogenizer, and centrifuged. [^{3}H]noradrenaline was adsorbed onto alumina at pH 8·4, eluted from it with 0·25 N HCl and counted by liquid scintillation spectrometry as already described in detail.[7]

Rat brain. 34276-Ba (100 mg/kg) or desmethylimipramine (25 mg/kg) was administered orally once or daily for either 3 or 11 days. 1·5 hr after the (last) administration, the rats received an intracisternal injection of [^{3}H]noradrenaline (4 μc/20 μl/rat), according to the method of Schanberg *et al.*[10] The whole brains were removed 1 hr later, and the content of [^{3}H]noradrenaline was estimated as described above.

Cat organs. Cats of both sexes (2·2–3·6 kg body weight) were used. Oral treatments were carried out by stomach tubing of both drugs dissolved in physiological saline. The cats were anaesthetized with sodium pentobarbital (Nembutal^R, 35 mg/kg, intraperitoneally). Trachea and jugular vein were cannulated. For intravenous treatments, the drugs were injected into the jugular vein and administration lasted exactly 1 min. Drug solutions were made in a mixture of polyethylene glycol 400 and physiological saline 1·5:9. DL-[^{3}H]noradrenaline (9·71 c/mM, 50 μc/ml/kg) was infused at a rate of 0·15 ml/min using a constant rate infusion pump (Unita 1, Type 1830, Braun, Melsungen, Germany). Infusions were started 20 min or 2 hr after intravenous or oral drug treatment, respectively. One hr after the beginning of the infusion the cats were exsanguinated. The organs were removed and prepared for [^{3}H]noradrenaline uptake estimations as described for rat organs.

Chick organs. The estimation of [^{3}H]noradrenaline uptake in heart and brain was performed first in pilot studies without drug pretreatment, using 1 day (16–24 hr), or 4-, 8- and 12-day-old chicks, respectively. The influence of 34276-Ba or of desmethyl-

imipramine was determined only in 1-day-old chicks (30–40 g body weight). The drugs were injected intraperitoneally (0·1 ml/chick) 1 hr before an intravenous injection of DL-[^{3}H]noradrenaline (8·45 c/mM, 20 μc/0·1 ml/chick). The organs were removed 1 hr later for uptake estimations as described for rat organs.

Uptake of [^{3}H]metaraminol in the rat heart

The test substances were administered 2 hr before an intravenous injection of DL-[^{3}H]metaraminol (6·5 c/mM, New England Nuclear, Boston, Mass., U.S.A., 200 μc/ml/kg body weight). The rats were sacrificed 30 or 180 min after [^{3}H]metaraminol, according to the procedure of Obianwu.[11] [^{3}H]metaraminol in the heart was estimated after chromatography on Dowex 50 W $\times$ 4 columns.[12]

Concentrations of endogenous noradrenaline

Noradrenaline was extracted in 10% trichloroacetic acid, adsorbed onto alumina at pH 8·4 and eluted from it with 0·25 N HCl, as described above for [^{3}H]noradrenaline uptake experiments. The content of noradrenaline in the eluates was determined fluorometrically, according to the trihydroxyindole procedure of von Euler and Lishajko,[13] but using 10 N NaOH rather than 5 N NaOH for the formation of noradrenolutine. The recovery of noradrenaline added to heart or brain homogenates averaged 84·7 per cent (n = 9). No correction for incomplete recovery has been made.

RESULTS

Catecholamine content in the rat heart and brain

In a first series of experiments, the effect of 34276-Ba on the endogenous catecholamine content of the rat heart and brain was studied. Noradrenaline in heart and brain and dopamine in the brain were estimated after either a single administration or a repeated treatment. The results shown in Table 1 indicate that the drug has no significant influence on catecholamine content in heart and brain.

The data of the 3 days treatment in Table 1 are compiled from four separate experiments. In two other experiments (not included in the Table) a small but significant ($P < 0·05$) decrease in the noradrenaline content of heart (−17 per cent) and brain (−14 per cent) was found, but the dopamine content of the brain remained unaffected. The reason for this inconsistent effect on the noradrenaline content in different experiments is at present unexplained.

Amine uptake in peripheral organs

Isolated bovine splenic nerve granules. The ability of 34276-Ba to alter [^{3}H]noradrenaline uptake into isolated nerve granules was compared with that of imipramine. Both drugs inhibited the uptake in a concentration dependent manner (Fig. 2). The ED_{50} of 34276-Ba was about 10^{-4} M. Imipramine was about 3-fold less potent. For both drugs, the concentrations needed to inhibit [^{3}H]noradrenaline uptake were of the same order of magnitude than those of other psychotropic drugs, such as chlorpromazine or benzoctamine, but they were at least 1000-fold higher than those of reserpine, under the same experimental conditions.[7]

TABLE 1. EFFECTS OF 34276-Ba (100 mg/kg) ON THE CATECHOLAMINE CONTENT OF RAT HEART AND BRAIN

	Heart		Brain			
	Noradrenaline		Noradrenaline		Dopamine	
	(μg/g)		(μg/g)		(μg/g)	
	Controls	34276-Ba	Controls	34276-Ba	Controls	34276-Ba
A. Single treatment						
2·5 hr	1·03 ± 0·04 (6)	1·04 ± 0·03 (10)	0·341 ± 0·018 (6)	0·378 ± 0·014 (5)	0·660 ± 0·041 (6)	0·686 ± 0·052 (5)
4 hr		1·02 ± 0·08 (4)		0·364 ± 0·021 (4)		0·645 ± 0·062 (4)
B. Repeated treatment						
3 days	0·82 ± 0·04 (14)	0·79 ± 0·03 (14)	0·407 ± 0·021 (12)	0·427 ± 0·012 (12)	0·680 ± 0·032 (12)	0·650 ± 0·024 (12)
11 days	0·74 ± 0·04 (9)	0·82 ± 0·07 (12)	0·394 ± 0·017 (6)	0·335 ± 0·023 (9)	0·655 ± 0·031 (6)	0·622 ± 0·017 (9)

The drug was administered orally. For repeated daily treatment the organs were removed 2 hr after the last administration. Figures represent mean values ± S.E. of the extract number shown in parentheses. The differences between control and treated groups are not statistically significant ($P > 0·05$).

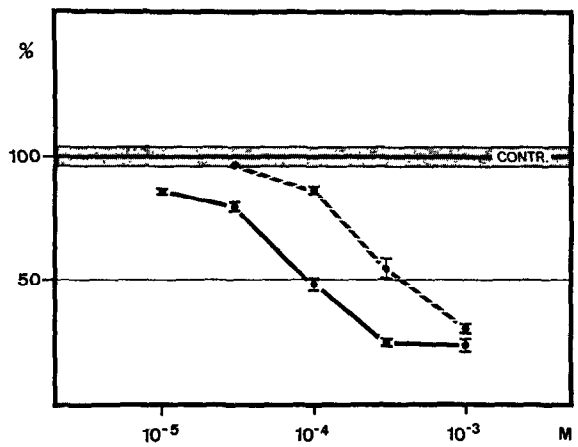

FIG. 2. Effect of 34276-Ba on the uptake of [^{3}H]noradrenaline into isolated bovine splenic nerve granules. Comparison with imipramine. Isolated bovine splenic nerve granules were incubated at 37° for 20 min with 0·1 mM DL-[^{3}H]noradrenaline (0·12 μc/μg) in a medium containing 0·003 M ATP-$MgCl_2$. The drugs were not preincubated. Symbols represent the mean values ± S.E. of four to six determinations. The symbol, where no standard deviation is given, represents the mean value of two determinations. Abscissa: molar concentration of drugs. Ordinate: [^{3}H]noradrenaline taken up, expressed as percentage of control values (n = 6).

——— 34276-Ba.
- - - - - Imipramine.

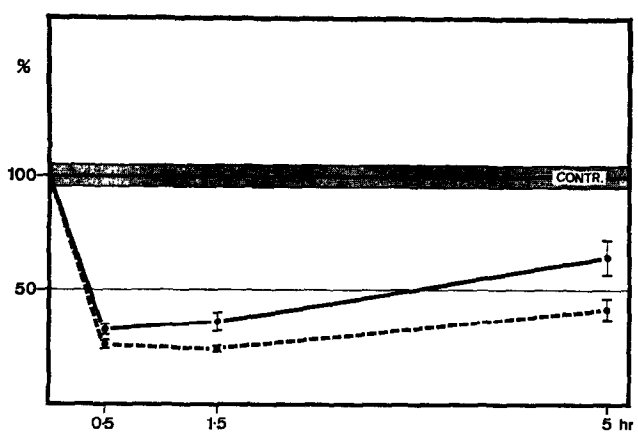

FIG. 3. Effect of an intravenous injection of 34276-Ba (10 mg/kg) or of imipramine (10 mg/kg) on the uptake of [^{3}H]noradrenaline in the rat heart. Rats received an intravenous injection of [^{3}H]-noradrenaline (100 μc/kg) at different times after drug pretreatment. They were sacrificed 1 hr after the injection of [^{3}H]noradrenaline. Abscissa: time elapsing between drug and [^{3}H]noradrenaline injections. Ordinate: [^{3}H]noradrenaline taken up, expressed as percentage of control values. The symbols represent mean values ± S.E. of four or five experiments. The absolute values ± S.E. of eight control experiments were 95·7 ± 4·7 counts/min . 10^3/g heart.

——— 34276-Ba.
- - - - - Imipramine.

Rat heart. The time course of the inhibitory effect of 34276-Ba on noradrenaline uptake was studied and compared with that of imipramine, either after single intravenous injection of after single oral administration of both drugs. The results show that an intravenous dose of 10 mg/kg of either drug causes a strong and long lasting inhibition of noradrenaline uptake (Fig. 3). The degree of inhibition was somewhat smaller after treatment with 34276-Ba than after imipramine. A longer duration of action was seen after imipramine. Oral administration also produced a strong and long lasting inhibition of noradrenaline uptake with both drugs (Fig. 4). In contrast to the effects seen after intravenous injection, the potency of 34276-Ba was 1/3 to 1/10 that of imipramine in the latter case.

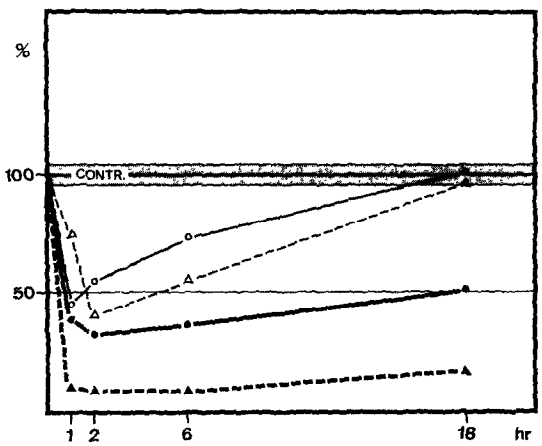

FIG. 4. Effect of an oral administration of 34276-Ba or of imipramine on the uptake of [^3H]noradrenaline in the rat heart. Legend as in Fig. 3. The symbols represent mean values of two experiments. The absolute values ± S.E. of seven control experiments were 110·5 ± 5·0 counts/min . 10^3/g heart.

● —— ● 34276-Ba 100 mg/kg.
○ —— ○ 34276-Ba 30 mg/kg.
▲ - - - ▲ Imipramine 30 mg/kg.
△ - - - △ Imipramine 10 mg/kg.

From these time-course experiments it was considered that a pretreatment time of 2 hr would be appropriate to study dose–response relationships after oral and subcutaneous treatment. For intravenous injections a pretreatment time of 30 min was chosen. Thus, in a second series of experiments, the effects of various doses were determined at these fixed pretreatment times. The dose–response curves after oral, subcutaneous or intravenous treatment with 34276-Ba or with imipramine are shown in Figs. 5 and 6.

The mean ED_{50} of both drugs were determined graphically. For imipramine, they were 4·4, 6·0 and 7·3 mg/kg after intravenous, subcutaneous, and oral administrations, respectively. The steep slope of the curve obtained from oral treatment is of particular interest as well as the fact that after subcutaneous injection small doses are more active and high doses less active than after oral treatment. The small shift between the various routes of administration reflects the high resorption rate of imipramine. By contrast, the dose–response curves of 34276-Ba can be clearly distinguished, according

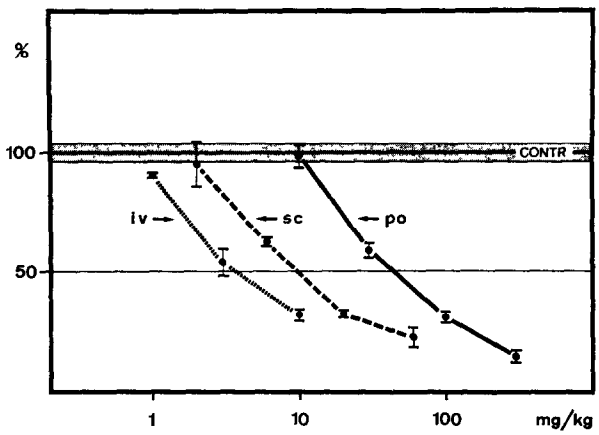

FIG. 5. Effect of 34276-Ba on the uptake of [^{3}H]noradrenaline in the rat heart. Dose–response relationships after intravenous (i.v.), subcutaneous (s.c.) and oral (p.o.) treatment. Rats received an intravenous injection of [^{3}H]noradrenaline (100 μc/kg) either 30 min (i.v. experiments) or 2 hr (s.c. and p.o. experiments) after drug treatment. They were sacrificed 1 hr later. Abscissa: dose of 34276-Ba. Ordinate: [^{3}H]noradrenaline taken up, expressed as percentage of controls. The symbols represent mean values ± S.E. of four to nine experiments. The absolute values ± S.E. of 16 control experiments were 107·1 ± 4·1 counts/min. 10^3/g heart.

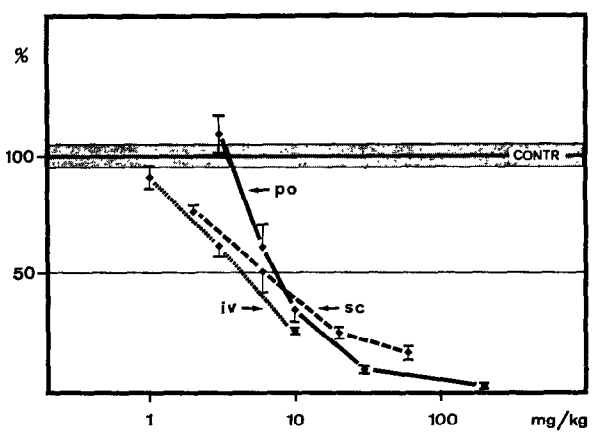

FIG. 6. Effect of imipramine on the uptake of [^{3}H]noradrenaline in the rat heart. Dose–response relationships after intravenous (i.v.), subcutaneous (s.c.) and oral (p.o.) treatment. Experimental conditions: see legend of Fig. 5. Abscissa: dose of imipramine. Ordinate: [^{3}H]noradrenaline taken up, expressed as percentage of controls. The symbols represent mean values ± S.E. of three to eleven experiments. The absolute values of 10 control experiments were 107·5 ± 4·6 counts/min. 10^3/g heart.

to the route of administration. The mean ED_{50} were 3·7, 9·7 and 44 mg/kg for intravenous, subcutaneous, and oral treatment, respectively. The three curves have a very similar slope indicating that 34276-Ba was respectively about 2·5 times and 10 times less potent after subcutaneous or oral treatment than after intravenous injection.

Since desmethylimipramine is considered to be a more active metabolite, it was used as another reference drug in several test systems, a dose–response curve was also carried out after oral treatment with this drug. It showed a threshold dose about 2·5

mg/kg and inhibitions of 64·8 ± 4·0 per cent after 6 mg/kg ($n = 13$) and of 93·7 ± 1·0 per cent after 15 mg/kg ($n = 3$). The ED_{50} was 4·8 mg/kg. Thus the slopes of the dose–response curves were similar for desmethylimipramine and imipramine.

In addition to cocaine, several antidepressant drugs have been shown to inhibit the guanethidine-induced depletion of noradrenaline stores[14–16] as well as other effects of guanethidine.[17,18] These substances have the common property to inhibit noradrenaline uptake into sympathetic nerve endings. They inhibit obviously also the uptake of guanethidine by the same mechanism, thus reducing its potency.[19] Since 34276-Ba is a potent inhibitor of noradrenaline uptake, it was of interest to substantiate a presumable interaction with guanethidine at the level of the adrenergic sympathetic neurons.

A single dose of 34276-Ba reduced the noradrenaline depletion caused by various doses of guanethidine so that the dose–response curve of guanethidine alone showed a parallel shift toward the controls (Fig. 7). Similarly, the noradrenaline depleting

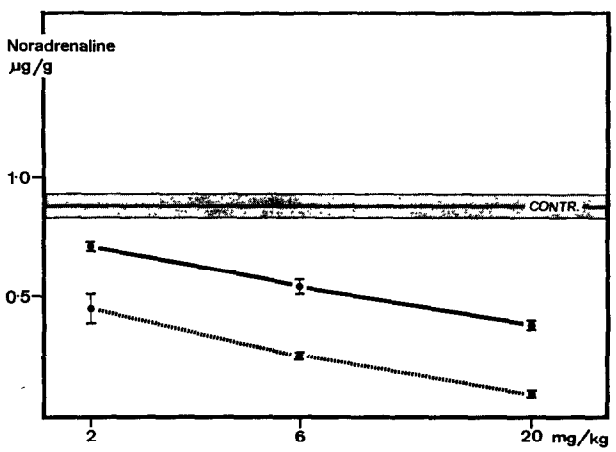

FIG. 7. Effect of 34276-Ba on the guanethidine-induced noradrenaline depletion in the rat heart. Rats were treated with 34276-Ba (100 mg/kg, orally) 30 min before a subcutaneous injection of guanethidine. They were sacrificed 24 hr after guanethidine treatment. Abscissa: dose of guanethidine. Ordinate: noradrenaline content of the heart. The symbols represent mean values ± S.E. of three to six extracts. Nine determinations were carried out in the heart of vehicle-treated controls.
----- Guanethidine alone.
——— 34276-Ba + guanethidine.

effect of a repeated treatment with a fixed dose of guanethidine was reduced in a dose-dependent manner by 34276-Ba (Table 2). In this experiment, the rats were treated daily for 4 days with either guanethidine alone (60 mg/kg/day, p.o.) or guanethidine following a pretreatment with 20 or 60 mg/kg/day, s.c. of 34276-Ba. The content of noradrenaline in the heart of rats treated with 34276-Ba alone in each set of experiment did not differ significantly ($P > 0{\cdot}05$) from that found in the NaCl treated controls.

In another series of experiments the uptake of [^{3}H]noradrenaline was measured 3, 10 or 30 min after administration of the labelled amine to rats pretreated with 34276-Ba or desmethylimipramine. The content of [^{3}H]noradrenaline in the plasma of the same animals was also determined. As shown in Table 3, the inhibitions of

TABLE 2. EFFECT OF 34276-Ba ON THE GUANETHIDINE-INDUCED NORADRENALINE DEPLETION IN THE RAT HEART

Treatment	*n*	Noradrenaline (μg/g)
NaCl	8	0·90 ± 0·046
Guanethidine 60 mg/kg day × 4, p.o.	3	0·18 ± 0·012
34276-Ba 20 mg/kg day × 4, s.c. 30 min before guanethidine	3	0·29 ± 0·031*
34276-Ba 60 mg/kg day × 4, s.c. 30 min before guanethidine	3	0·50 ± 0·020†

Rats received 60 mg/kg guanethidine daily for 4 days, either alone or after treatment with 34276-Ba at a dose of 20 or 60 mg/kg daily. The hearts were removed 2 hr after the last dose of guanethidine.

n = number of extracts of two hearts each.

* 0·01 < P < 0·05
† P < 0·001
} versus guanethidine alone.

uptake in the heart were the same after 10 and 30 min and corresponded to that observed in the standard experiments (60 min after [3H]noradrenaline) described above. After 3 min, however, the degree of inhibition of [^{3}H]noradrenaline uptake was much less pronounced ($P<0·01$). This was true for either 34276-Ba or desmethylimipramine.

The amounts of [^{3}H]noradrenaline found in the plasma in these experiments were in each case scarcely higher in the drug treated rats than in the controls. The differences were not significant ($P>0·05$).

Uptake and release of [^{3}*H*]*metaraminol.* Two different amine uptake-concentration mechanisms of the adrenergic neurons have been described: the transport process through the nerve cell membrane and the uptake retention mechanism in the intracellular storage granules.[20,21] Metaraminol seems to utilize similar transport and storage processes to noradrenaline with the particularity that it is not destroyed by monoamine oxidase or catechol-*O*-methyl-transferase (see Carlsson and Waldeck[22]). By estimating the amounts of [^{3}H]metaraminol in the heart 30 min and 3 hr after its administration, it is possible to distinguish which of the two uptake-concentration mechanisms is affected by a given drug.[11]

The amount of [^{3}H]metaraminol present in the heart 30 min and 3 hr after its administration was thus determined in rats pretreated with 34276-Ba. Reserpine and imipramine were used as reference drugs. The results, shown in Table 4, clearly indicate that the initial uptake of [^{3}H]metaraminol (30 min after its injection) was reduced after all three drugs. The inhibition caused by reserpine was 40–45 per cent, whereas the effects of imipramine and 34276-Ba were stronger and both of the same order of magnitude (about 70 per cent). Three hr after [^{3}H]metaraminol only reserpine showed a diminished retention of the [^{3}H]amine, indicating a profound impairment of the intracellular storage capacity. On the contrary, the [^{3}H]metaraminol retention was not altered by either imipramine or 34276-Ba.

TABLE 3. EFFECT OF DESMETHYLIMIPRAMINE (DMI) AND 34276-Ba ON THE UPTAKE OF [^{3}H]NORADRENALINE IN THE RAT HEART AND ON THE DISAPPEARANCE OF [^{3}H]NORADRENALINE FROM THE CIRCULATION

Time between [^{3}H]-noradrenaline injection and killing (min)	Controls		DMI		34276-Ba	
	Plasma (counts/min $.10^3$/ml)	Heart (counts/min $.10^3$/g)	Plasma (counts/min $.10^3$/ml)	Heart (counts/min $.10^3$/g)	Plasma (counts/min $.10^3$/ml)	Heart (counts/min $.10^3$/g)
3	17·5 ± 1·8	136·5 ± 3·0	20·5 ± 1·0	87·3 ± 2·7	19·3 ± 1·2	88·0 ± 8·8
10	7·3 ± 0·41	130·6$^{N.S.}$ ± 4·0	8·1 ± 0·32	50·8* ± 4·6	8·2 ± 0·40	45·9* ± 2·5
30	3·2 ± 0·2	131·1 ± 5·6	3·4 ± 0·12	50·7 ± 3·1	3·5 ± 0·15	38·2 ± 7·8

DMI (6 mg/kg, p.o.) and 34276-Ba (100 mg/kg, p.o.) were administered 2 hr before the injection of [^{3}H]noradrenaline (100 μc/ml/kg, i.v.).
Figures represent the mean values ± S.E. of four to six extracts.
N.S. = not significant ($P > 0.05$)
* $0.001 < P < 0.01$
} as compared with 3 min values.

TABLE 4. EFFECTS OF IMIPRAMINE, RESERPINE AND 34276-Ba ON THE UPTAKE AND RELEASE OF [^{3}H]METARAMINOL IN THE RAT HEART

Treatment	n	[^{3}H]metaraminol counts/min.10^3/g	
		30 min	180 min
NaCl	15	328 ± 9	303 ± 14
Imipramine	6	97 ± 7	97 ± 12
Reserpine	12	189 ± 12	45 ± 6
34276-Ba	6	102 ± 11	88 ± 11

Imipramine (10 mg/kg, p.o.), reserpine (1 mg/kg, s.c.) and 34276-Ba (100 mg/kg, p.o.) were administered 2 hr before the injection of [^{3}H]metaraminol. [^{3}H]metaraminol (200 μc/ml/kg) was injected intravenously 30 min or 180 min before removing the organs.

n = number of extracts of two hearts each.

The 180 min values differ significantly from the 30 min values only in the reserpine-treated group ($P < 0.001$).

Rat: other peripheral organs. The effect of 34276-Ba on [^{3}H]noradrenaline uptake was also studied in other peripheral organs having a rich adrenergic innervation. The experiments were carried out with a dose producing inhibition in the heart and at the pretreatment time chosen for establishing dose–response curves in this organ. The noradrenaline uptake was determined in spleen, salivary gland and vas deferens. The result showed no inhibition in the spleen, a pronounced inhibition (about 50 per cent) in the salivary gland and an increased uptake in the vas deferens (Table 5). These results are qualitatively and quantitatively similar to that obtained after pretreatment with desmethylimipramine at a dose (6 mg/kg, p.o.) inhibiting the noradrenaline uptake in the heart to the same extent as the dose of 34276-Ba used here.[23]

TABLE 5. EFFECT OF 34276-Ba ON THE UPTAKE OF [^{3}H]NORADRENALINE IN THE RAT SPLEEN, SALIVARY GLANDS AND VAS DEFERENS

Organ	[^{3}H]noradrenaline counts/min.10^3/g tissue	
	Control $n = 5$	34276-Ba $n = 8$
Spleen	10·1 ± 0·5	10·6 ± 0·8
Salivary glands	64·2 ± 3·4	33·1 ± 1·6*
Vas deferens	15·8 ± 1·8	25·7 ± 1·5†

[^{3}H]noradrenaline (100 μc/ml/kg) was injected intravenously 2 hr after the administration of 34276-Ba (100 mg/kg, p.o.) and 1 hr before removing the organs, respectively.

n = number of extracts. The organs of two rats were pooled for one extract.

* $P < 0.001$.

† $0.001 < P < 0.01$.

Cat organs. 34276-Ba was administered orally or intravenously before an intravenous infusion of [^{3}H]noradrenaline, and the uptake of labelled amine was estimated in atria, ventricles (the remainder of the heart), spleen and salivary glands as described

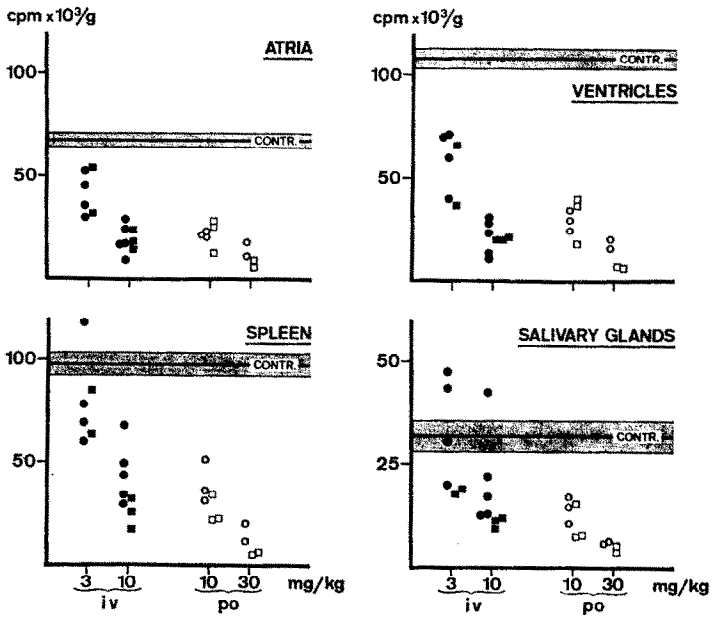

FIG. 8. Effects of 34276-Ba and of imipramine on the uptake of [³H]noradrenaline in cat organs. [³H]noradrenaline (50 μc/ml/kg body weight) was infused into the jugular vein 20 min or 2 hr after intravenous or oral drug treatment, respectively, as described under "Materials and Methods". The organs were removed 1 hr after the beginning of the infusion (i.e. 35–45 min after the end of the infusion) for the determination of endogenous and radioactive noradrenaline. Each symbol represents a single organ. Circles correspond to the 34276-Ba-treated cats and squares to the imipramine-treated cats. Control values were obtained from ten cats for atria and ventricles and from nine cats for spleen and salivary glands.

in "Materials and Methods". Cats treated with similar doses of imipramine were used for comparison. The results are shown in Fig. 8.

The ability of 34276-Ba to inhibit strongly the uptake of [³H]noradrenaline into adrenergically innervated tissues was corroborated by these experiments in another animal species.

No obvious quantitative or qualitative difference between the inhibiting potencies of 34276-Ba and imipramine could be noted, and oral treatment seemed as effective as intravenous injection. In contrast to the results obtained in the rat, there was no difference in the cat between imipramine and 34276-Ba with respect to oral resorption. The inhibition of [³H]noradrenaline uptake was dose-dependent in all four organs. It was slightly but regularly more pronounced in the ventricle than in the atria. In contrast to the rat, the degree of inhibition proved to be of the same order of magnitude in the spleen and in the atria. Finally, the effects were less marked in the salivary glands than in the other tissues studied. Since [³H]noradrenaline uptake partially depends on the richness of adrenergic innervation,[24] the content of endogenous noradrenaline was also determined in these organs, and specific activity (counts/min [³H]noradrenaline/μg noradrenaline) was calculated (Table 6). The results show again a dose-dependent reduction of the specific activity and present thus further evidence of an effective inhibition of noradrenaline uptake into peripheral adrenergic nerve terminals.

TABLE 6. EFFECTS OF IMIPRAMINE AND 34276-Ba ON SPECIFIC ACTIVITY OF [^{3}H]NORADRENALINE IN CAT ORGANS

Treatment (mg/kg)	Specific activity (counts/min.10^3 [^{3}H]NA/μg NA)			
	Ventricles	Atria	Salivary glands	Spleen
NaCl	81·6 ± 7·2	51·5 ± 4·9	26·0 ± 3·7	48·8 ± 8·4
	$n = 10$	$n = 10$	$n = 9$	$n = 9$
Imipramine	28·0	27·9	12·1	27·6
3 i.v. ($n = 2$)	49·7	38·3	18·6	14·8
10 i.v. ($n = 3$)	15·8‡ ± 0·5	13·6† ± 1·2	8·6* ± 1·0	12·6* ± 1·7
10 p.o. ($n = 3$)	19·8† ± 10·2	18·7† ± 10·7	7·3* ± 2·3	11·7* ± 3·7
30 p.o. ($n = 2$)	4·3	4·4	3·9	3·6
	4·7	5·3	3·5	2·4
34276-Ba	36·4† ± 3·9	31·1* ± 5·5	24·9 ± 3·3	35·2 ± 0·4
3 i.v. ($n = 4$)			N.S.	N.S.
10 i.v. ($n = 5$)	20·9‡ ± 1·8	18·5† ± 1·0	14·9 ± 2·7	20·3* ± 1·8
			N.S.	
10 p.o. ($n = 3$)	20·3‡ ± 4·4	16·4† ± 3·8	7·8* ± 0·4	16·5* ± 1·0
30 p.o. ($n = 2$)	9·8	8·3	5·3	7·5
	9·1	8·8	5·2	10·5

The specific activities were calculated from the extracts shown in Fig. 8. The absolute noradrenaline contents in the organs of control cats were: 1·48 ± 0·17 μg/g in the ventricles, 1·33 ± 0·11 μg/g in the atria, 1·33 ± 0·08 μg/g in the salivary glands and 2·02 ± 0·32 μg/g in the spleen.

The single values are shown when only two experiments have been performed. The other values represent means ± S.E.

* $0{\cdot}01 < P < 0{\cdot}05$.

† $0{\cdot}001 < P < 0{\cdot}01$.

‡ $P < 0{\cdot}001$.

Brain

Rat. [^{3}H]norepinephrine injected into the lateral ventricles or into the cisterna magna mixes with endogenous norepinephrine pools and seems to be a suitable tracer of this amine.[25,26] The effect of 34276-Ba on uptake of intracisternally administered [^{3}H]noradrenaline was compared with that of desmethylimipramine. The drugs were administered either once, or daily for 3 days, or daily for 11 days. The results show that both drugs are able to reduce [^{3}H]noradrenaline uptake in the rat brain (Fig. 9). This effect was cumulative with either 34276-Ba or desmethylimipramine. The onset of the inhibiting action was slower with 34276-Ba than with desmethylimipramine as shown by the small and not significant decrease in uptake 1·5 hr after a single treatment with 34276-Ba, contrasting with the significant ($P < 0{\cdot}05$) inhibition caused under the same conditions by desmethylimipramine. After 3 or 11 days treatment with 34276-Ba, the decrease in [^{3}H]noradrenaline uptake became evident. It was smaller, however, than that seen after similar treatment with desmethylimipramine.

With both drugs, the magnitude of the inhibition was much less than that seen in the heart.

Chick. It was previously reported[27] that in the chick the blood–brain barrier does not develop until some time after hatching. This finding has been substantiated by estimating the amount of intravenously injected [^{3}H]noradrenaline taken up by this organ in the course of the first days after hatching,[28] where it was found that in 4-day-old chicks the [^{3}H]noradrenaline uptake is 2·5–3 times less than in 16–24-hr-old animals. Figure 10 shows that the uptake capacity progressively diminishes so that it

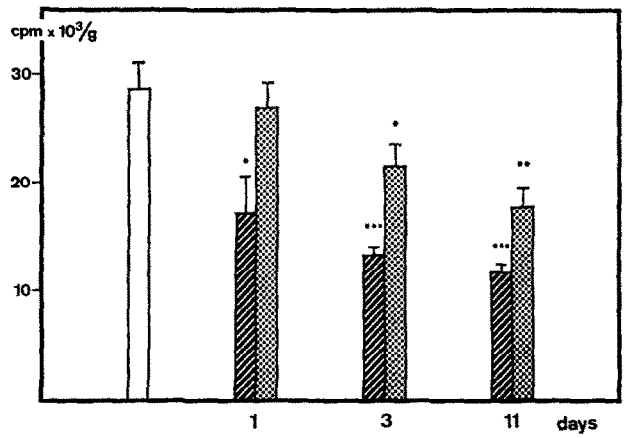

FIG. 9. Effects of 34276-Ba and of desmethylimipramine on the uptake of [³H]noradrenaline in the rat brain. Rats were treated once daily with 34276-Ba (100 mg/kg, orally) or with desmethylimipramine (25 mg/kg, orally). 1·5 hr after the (last) administration, they received an intracisternal injection of [³H]noradrenaline (4 μc/20 μl/rat). The brains were removed 1 hr later for [³H]noradrenaline estimations. Columns represent mean values and S.E. of five extracts from treated rats and of six controls. Open columns: controls. Hatched columns: desmethylimipramine. Dotted columns: 34276-Ba.

* 0·01 < P < 0·05.
† 0·001 < P < 0·01.
‡ < P < 0·001.

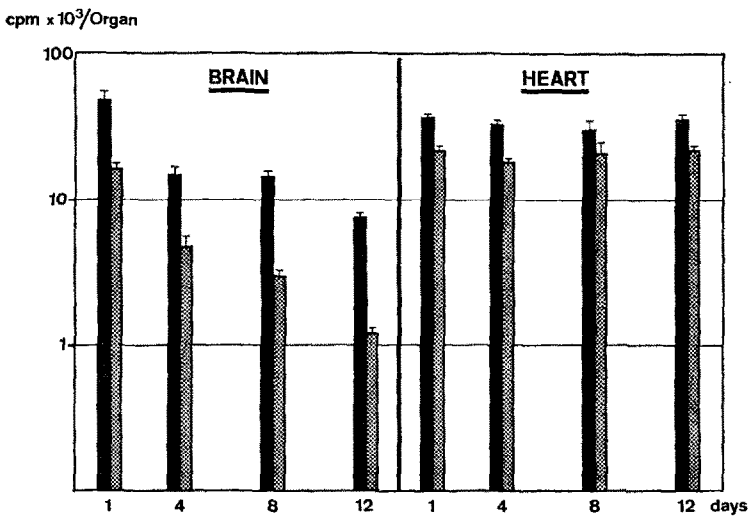

FIG. 10. [³H]noradrenaline uptake in brain and heart of 1–12-day-old chicks. [³H]noradrenaline (20 μc/0·1 ml/chick) was injected intravenously 1 hr before removing the organs. Columns represent mean values and S.E. of 12 (1 day) or 5 (4, 8 and 12 days) determinations. Hatched columns: total radioactivity. Dotted columns: [³H]noradrenaline.

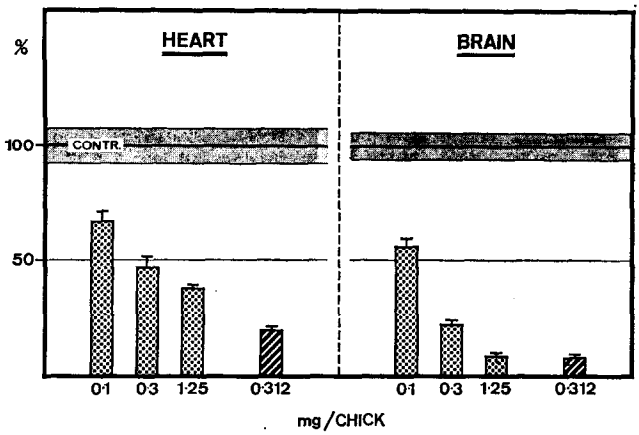

FIG. 11. Effect of 34276-Ba on the uptake of [^{3}H]noradrenaline in chick heart and brain. [^{3}H]-noradrenaline (20 μc/0·1 ml/chick) was injected intravenously 1 hr after intraperitoneal drug treatment and 1 hr before removing the organs, respectively. Columns represent mean values and S.E. of seven to nine experiments, expressed as percentage of the controls. The absolute mean values $\pm$ S.E. of 12 controls were: 13·4 $\pm$ 1·1 counts/min. 10^3/brain and 22·3 $\pm$ 1·4 counts/min. 10^3/heart. Dotted columns: 34276-Ba. Hatched columns: Desmethylimipramine.

was reduced by more than 90 per cent in 12-day-old chicks. In the heart of the same animals, however, there was no difference in the magnitude of [^{3}H]noradrenaline uptake during this time.

According to this observation, 16–24-hr-old chicks were used to study the influence of various doses of 34276-Ba on the [^{3}H]noradrenaline uptake in the chick brain. One dose of desmethylimipramine was assayed as a reference and the hearts of these animals were also removed and analysed for [^{3}H]noradrenaline in order to get a direct comparison of the inhibiting effects in the brain and in a peripheral organ in this animal species. 34276-Ba produced a strong dose-dependent inhibition of [^{3}H]-noradrenaline in both organs (Fig. 11). In each case, inhibition was more marked in the brain than in the heart. This was also observed after desmethylimipramine treatment. Thus the degree of relative inhibition in chick heart and brain differed fundamentally from that found in rat heart and brain.

DISCUSSION

The chemical structure of 34276-Ba resembles very closely that of another new psychotropic drug, benzoctamine (TACITIN^R).[5] Interestingly, this study and earlier investigations[7] show that the profiles of the biochemical effects of both dibenzo-bicyclo-octadiene derivatives can be clearly differentiated.

The results of the present study demonstrate that 34276-Ba is a powerful inhibitor of noradrenaline uptake into several sympathetically innervated tissues. The mechanism by which this inhibition takes place may be considered primarily as an impairment of the nerve cell "membrane pump" to take up noradrenaline from the synaptic region and to transport it into the nerve cell cytoplasma where it can be retained by the intracellular storage granules. At quite high concentrations 34276-Ba inhibited noradrenaline uptake in isolated splenic nerve granules. This effect was not observable

in vivo, under the conditions used in this study, since the subcellular retention of metaraminol was not impaired and since no pronounced decreases of the endogenous noradrenaline content were detected. However, the fact that a small decrease (15–20 per cent) of the noradrenaline content has been observed in some experiments indicates that an intraneuronal site of action of 34276-Ba cannot be completely excluded.

The ability to inhibit the uptake process as the level of the neuronal membrane is a characteristic of tricyclic antidepressants such as imipramine, desmethylimipramine or amitriptyline. Through the same process, 34276-Ba most probably inhibits the uptake of guanethidine into the adrenergic neurons.

The characteristics of uptake inhibition by 34276-Ba are very similar to those of imipramine or desmethylimipramine. The differences seen between the effects of oral administrations and intravenous injections in rats and cats are obviously dependent on differences in resorption between the two animal species.

The experiments, where blood and heart were removed 3, 10, or 30 min after the injection of [^{3}H]noradrenaline have shown that the inhibitions of uptake caused by 34276-Ba or desmethylimipramine are much less marked after 3 min than after 10 or 30 min. This suggests that the rat heart is able to take up quite large quantities of noradrenaline under the influence of such "uptake inhibitors". In fact, the comparison of the values obtained at different times indicate that an important part of the [^{3}H]-noradrenaline taken up by the cardiac tissue immediately after its injection can be washed out within the first few minutes. Then the amount of cardiac [^{3}H]noradrenaline reaches a plateau corresponding probably to the degree of neuronal uptake. This means that the binding sites involved in this immediate uptake obviously retain noradrenaline less firmly than the uptake sites which are selectively blocked by these drugs. Interestingly, Weiner and Trendelenburg[29] reported that 2 min after an injection of [^{14}]adrenaline to rats, the radioactivity of the heart was not affected by pretreatment with cocaine, and Bhagat *et al.*[30] found that cocaine did not inhibit the uptake of [^{3}H]noradrenaline into isolated guinea-pig atria when the tissue was incubated with the labelled amine for only 1 min.

The degrees of noradrenaline uptake inhibition in various cat organs were not identical to those in the corresponding rat organs. This is probably due to species differences in the uptake processes into the organs rather than to a preferential distribution of the drug, for the differences in the inhibiting effects of 34276-Ba in the cat and in the rat were shared by imipramine and by desmethylimipramine, respectively.

The mechanism of noradrenaline release and reuptake described for peripheral tissues operates likely also in the brain. It was thus essential to estimate the influence of 34276-Ba on the central uptake processes. The experiments carried out by introducing [^{3}H]noradrenaline into the rat brain by an intracisternal injection as well as the experiments on chicks have shown that 34276-Ba markedly inhibits noradrenaline uptake in the brain. Therefore, 34276-Ba might allow more free noradrenaline released from central sympathetic neurones to act on central noradrenergic receptors. Consequently, the drug might potentiate noradrenaline effects at central adrenergic synapses. The mechanism of action of the new antidepressant drug at the biochemical level resembles that described by Glowinski and Axelrod[31] for imipramine, desmethylimipramine or amitriptyline. These authors observed an inhibition of the uptake of [^{3}H]noradrenaline injected in the lateral ventricle of rats pretreated with these drugs. It is noteworthy that the degree of inhibition they reported for desmethylimipramine

agrees well with our observations gained from experiments when the [^{3}H]noradrenaline was administered intracisternally.

With both 34276-Ba and desmethylimipramine, a profound difference was observed between the degrees of inhibition in the rat and chick brain. In the chick brain, noradrenaline uptake was inhibited by 90–95 per cent after a single drug treatment whereas the maximal inhibitions found in the rat brain were 50–60 per cent even after repeated drug administration. The comparison of these effects with those observed in the heart showed that in the chick both drugs inhibited noradrenaline uptake in the brain more than in the heart whereas the inverse relation was found in the rat. This indicates a more effective blood–brain barrier for 34276-Ba and desmethylimipramine in the rat than in the chick. Alternatively, the noradrenaline uptake processes might be influenced differently at the level of the sympathetic neurones in heart and brain and furthermore the effect of an inhibitory substance might express differently whether the noradrenaline reaches the neurons in the brain through the blood vessels or the cerebrospinal fluid. On the other hand, the estimation of [^{3}H]noradrenaline uptake and of its inhibition by drugs is not necessarily as accurate in the whole brain as in the heart, for dopaminergic and serotonergic neurons as well as noradrenergic neurons are able to take up noradrenaline whereas uptake inhibitions obviously vary according to the neuronal type.[32]

Thus, 34276-Ba and imipramine or desmethylimipramine qualitatively show the same effects in all parameters tested. Quantitative differences become apparent in some animal species which, however, depend on the route of application and the organ examined.

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Note added in proof—Experiments on brain slices, which are still in progress, have shown that 34276-Ba inhibits noradrenaline uptake also *in vitro*. 34276-Ba is about 10 times less potent than desmethylimipramine in these experiments.