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EFFECT OF AMEZINIUM ON THE RELEASE AND CATABOLISM OF ^{3}H-MONOAMINES IN BRAIN SLICES

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Abstract—Occipitocortical slices of rats were preincubated with [^{3}H]noradrenaline or [^{3}H]serotonin; striatal slices were preincubated with [^{3}H]dopamine. The outflow of ^{3}H-compounds was studied. In slices preincubated with [^{3}H]noradrenaline, 1 nM amezinium diminished the outflow of deaminated metabolites (mainly [^{3}H]-3,4-dihydroxyphenylglycol). Higher concentrations in addition released [^{3}H]noradrenaline. The effects were antagonized by cocaine. In slices preincubated with [^{3}H]dopamine, 1 nM amezinium caused no change, but 10 nM amezinium decreased the outflow of deaminated products (mainly [^{3}H]-3,4-dihydroxyphenylacetic acid). Higher concentrations in addition released [^{3}H]dopamine. The effects were antagonized by nomifensine. In experiments with [^{3}H]serotonin, 1 nM amezinium caused no change, but 10 nM amezinium reduced the outflow of [^{3}H]-5-hydroxyindoleacetic acid. Higher concentrations in addition released [^{3}H]serotonin. The effects were antagonized by paroxetine. When amezinium was injected *in vivo* 1 hr before sacrifice, up to 10 mg/kg did not change the deamination of [^{3}H]noradrenaline in cortical cubes; amezinium (50 mg/kg) caused slight inhibition. The results indicate that amezinium, added *in vitro*, is a very potent inhibitor of the MAO inside cerebral noradrenergic neurones. It is less potent at dopaminergic and serotonergic neurones. When injected systemically *in vivo*, however, amezinium at moderate doses has no effect on central monoamine catabolism, probably because of exclusion from the brain by the blood–brain barrier.

Amezinium is a new compound which is now available for use in humans in the Fereral Republic of Germany. It increases the blood pressure by a peripheral indirect sympathomimetic action [1–4]. One component of this indirect action is a reversible inhibition of MAO† with selectivity for MAO A [4, 5]. When cell-free preparations are used and low concentrations of noradrenaline as substrate, amezinium is a rather weak inhibitor, with IC_{50} values ranging from 3 to 6 μM in crude homogenates or mitochondrial fractions of rat heart, rat liver or rabbit heart [4, 5]. In intact isolated tissues, however, amezinium is accumulated inside the postganglionic sympathetic neurones via the noradrenaline transport system and, hence, becomes a very potent inhibitor of the intraneuronal MAO, causing marked blockade at a concentration of 1 nM; extraneuronal MAO is inhibited only by much higher concentrations [5, 6]. ('Intraneuronal' refers to location inside, and 'extraneuronal' to location outside monoaminergic neurones.) Amezinium is a potent inhibitor of the intraneuronal MAO in peripheral tissues *in vivo* as well. When injected i.v. into rabbits, as little as 0.01 mg/kg reduce the MAO activity of the cardiac sympathetic nerves [5]. Direct evidence for pronounced accumulation in postganglionic sympathetic neurones was obtained with the help of [^{3}H]amezinium [7]. Inside the neurones, amezinium is stored in the synaptic vesicles from which it can be released by action potentials [7].

In animal experiments, amezinium had little effect on the central nervous system, although high doses (13–28 mg/kg p.o.) antagonized the reserpine-induced hypothermia and enhanced the central stimulatory effect of L-dopa in mice [8]. On the other hand, in healthy humans relatively low doses (10 or 20 mg total dose) elicited electroencephalographic and psychometric changes that resembled those produced by antidepressant drugs [9]. These effects might be due to an interference with MAO in brain. In brain homogenates, amezinium inhibits MAO only weakly, with IC_{50} values of 3.5 and 3.7 μM when low concentrations of noradrenaline and tryptamine, respectively, are used as substrates [4]. However, because of the accumulation mechanism demonstrated peripherally, the potency of amezinium in preparations containing intact cells might be much higher. Therefore, we studied its influence on the catabolism of monoamines in rat brain slices and, less extensively, in the rat brain *in vivo*.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 190–300 g.

In vitro. The animals were decapitated, and occipitocortical or striatal slices, 0.4 mm thick and weighing 3–10 mg, were prepared as described previously [10]. Occipitocortical slices were used for

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† Abbreviations used: COMT, catechol-*O*-methyl transferase (EC 2.1.1.6); DOMA, 3,4-dihydroxymandelic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPEG, 3,4-dihydroxyphenylglycol; DOPET, 3,4-dihydroxyphenylethanol; HIAA, 5-hydroxyindoleacetic acid; MAO, monoamine oxidase (EC 1.4.3.4); MOPEG, 3-methoxy-4-hydroxyphenylglycol; MTA, 3-methoxytyramine; NMN, normetanephrine; OMDA, *O*-methylated and deaminated metabolites; VMA, 3-methoxy-4-hydroxymandelic acid.

experiments with [^{3}H]noradrenaline and [^{3}H]serotonin, and striatal slices for experiments with [^{3}H]dopamine. Four or six occipitocortical slices were preincubated for 30 min at 37° in 2 ml of medium containing either 0.2 μM [^{3}H]noradrenaline (sp. act. 44.7 or 46.5 Ci/mmole) or 0.1 μM [^{3}H]serotonin (sp. act. 27.3 Ci/mmole). Ten to twelve striatal slices were similarly preincubated with 0.2 μM [^{3}H]dopamine (sp. act. 10.0 Ci/mmole). The slices were then washed three times with 3 ml medium each, transferred into glass superfusion chambers (one occipitocortical slice or two striatal slices per chamber) and superfused with [^{3}H]monoamine-free medium for 150 min at a rate of 1 ml/min. Samples (5, 10 or 30 min) of the superfusate were collected, starting after 50 min of superfusion. The superfusate of two chambers was always pooled. At the end of the experiment, the slices were solubilised in 0.5 ml Soluene 350 (Packard Instrument Co., Frankfurt, F.R.G.). The incubation and superfusion medium contained (mM): NaCl, 118.0; KCl, 4.8; $CaCl_2$, 1.3; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; Na_2EDTA, 0.03; ascorbic acid, 0.6; glucose, 8.1. The solution was saturated with carbogen (95% O_2, 5% CO_2) and prewarmed to 37°.

One millilitre of each superfusate was used for determinations of total tritium, whereas 9 ml were introduced into the separation procedure. [^{3}H]Noradrenaline and its metabolites were separated by the column chromatographic method of Graefe *et al.* [11]. The method permits separation of the following fractions: [^{3}H]noradrenaline, [^{3}H]DOPEG, [^{3}H]DOMA, [^{3}H]NMN and [^{3}H]OMDA. [^{3}H]Dopamine and its metabolites were separated by the column chromatographic method of Cubeddu *et al.* [12], which yields fractions of [^{3}H]dopamine, [^{3}H]DOPET, [^{3}H]DOPAC, [^{3}H]MTA and [^{3}H]OMDA. All results were corrected for incomplete recoveries and cross-contaminations.

For the separation of [^{3}H]serotonin from [^{3}H]HIAA, 5 mg Na_2EDTA, 6 mg NA_2SO_3, 25 μg unlabelled serotonin and 25 μg unlabelled HIAA were added to each 9 ml sample. The samples were poured on Dowex 50 WX4 columns (0.5 × 1.5 cm; treated as described by Graefe *et al.* [11]). The resin adsorbed serotonin, whereas HIAA passed through the columns. They were washed twice with 2 ml water each. Serotonin was eluted twice with 1 ml ethanol/6 M HCl, 1:1 (v/v). A 4 ml aliquot of the column effluent and the 2 ml of the eluate were counted for radioactivity. The recovery of serotonin (determined with [^{3}H]serotonin) was 81.7 ± 2.2% (n = 4); the recovery of HIAA (determined with unlabelled HIAA using the native fluorescence) was 100.3 ± 1.2% (n = 3). There was no consistent cross-contamination. All serotonin values were corrected for incomplete recovery.

The accuracy of the corrections was tested by comparing the total tritium measured in the samples with the sum of the tritium measured in the various fractions. In experiments with [^{3}H]noradrenaline, the measured total tritium amounted to 109.1 ± 0.4% of the sum of the measured fractions (n = 261); in experiments with [^{3}H]dopamine and [^{3}H]serotonin, the percentages were 92.2 ± 0.4 (n = 259) and 97.4 ± 0.3 (n = 288), respectively. Hence, there were only minor, albeit significant, differences from the theoretical 100% value.

In vivo. Two series of experiments were performed to study the effect of amezinium, injected *in vivo*. In one series, rats were decapitated 1 hr after i.p. injection of amezinium or tranylcypromine. Occipitocortical slices were prepared as described above and chopped into cubes, lengths of sides 0.4 mm. Portions of approximately 2 mg protein each were added to 1 ml medium, composed as above. [^{3}H]Noradrenaline, final concentration 0.01 μM, was added, and the mixture was incubated for 35 min under an atmosphere of 95% O_2 and 5% CO_2 at 37° in a shaking waterbath. One millilitre of ice-cold medium was then added, and particles were sedimentated in a refrigerated centrifuge. The pellet was washed once with 1 ml of medium. [^{3}H]DOPEG was determined in the combined supernatants as described by Graefe *et al.* [11]. The pellet was resuspended in medium, sedimented again and dissolved in 0.1 M NaOH. Part of it was used for determination of protein [13], another part for determination of total tritium accumulated in the tissue. Similar blanks for the [^{3}H]DOPEG formation were obtained, independently of whether pargyline 100 μM was added or boiled tissue cubes were used.

In the other series, rats were decapitated 2 hr after i.p. injection of amezinium or tranylcypromine. The whole brain minus cerebellum was homogenized in 5 ml 0.1 M $HClO_4$ containing 5 mg Na_2EDTA and 6 mg Na_2SO_3. Noradrenaline, dopamine and serotonin were quantified in the $HClO_4$ extracts without further purification by reverse-phase high pressure liquid chromatography with electrochemical detection. μ-Bondapak C18 columns (Waters) were used; the mobile phase was a mixture of 950 ml 0.05 M sodium acetate/0.03 M citric acid buffer, 50 ml methanol and 50 mg octylsulphate.

Chemicals. The following drugs were used: (−)-(ring 2, 5, 6)-[^{3}H]noradrenaline, NET 678, lot numbers 1271-176 and 1271-144 (N.E.N., Dreieich, F.R.G.); 5-(1, 2)-[^{3}H]hydroxytryptamine creatinine sulphate, NET 498, lot number 1537-047 (N.E.N., Dreieich, F.R.G.); (ring 2, 5, 6)-[^{3}H]dopamine, TRK. 284, batch 31 (Amersham Buchler, Braunschweig, F.R.G.). All ^{3}H-compounds were periodically checked for purity by column chromatography. Pargyline hydrochloride (Abbot, Ingelheim am Rhein, F.R.G.); amezinium methylsulphate (B.A.S.F., Ludwigshafen, F.R.G.); paroxetine hydrochloride (Ferrosan, Søborg, Sweden); nomifensine hydrogen maleate (Hoechst, Frankfurt, F.R.G.); cocaine hydrochloride (Merck, Darmstadt, F.R.G.); (±)-tranylcypromine sulphate (Röhm Pharma, Darmstadt, F.R.G.); 3,4-dihydroxy-2-methylpropiophenone, U-0521 (Upjohn, Kalamazoo, MI). Stock solutions of the drugs were prepared in water and diluted with the superfusion medium. Drugs for i.p. injections were dissolved in saline; doses refer to the salts.

Statistics. Means ± S.E.M. are given throughout. t-Tests were used to test differences between means and differences between a mean value and a theoretical value for significance. n, number of experiments.

RESULTS

Effects of amezinium added in vitro

Table 1 shows the pattern of ^{3}H-compounds in the outflow of tritium 55–60 min after preincubation of brain slices with [^{3}H]noradrenaline, [^{3}H]dopamine or [^{3}H]serotonin. In either case, the ^{3}H-monoamines contributed only about 10% to total tritium. In the case of [^{3}H]noradrenaline, [^{3}H]DOPEG and [^{3}H]OMDA were the major fractions; in the case of [^{3}H]dopamine, [^{3}H]DOPAC was the major compound, and in the case of [^{3}H]serotonin, [^{3}H]HIAA. Results obtained with pargyline and U-0521 support the validity of the separation procedure: for each neurotransmitter, the MAO inhibitor markedly reduced the outflow of deaminated products, whereas the COMT inhibitor strongly diminished the outflow of *O*-methylated compounds. The outflow of [^{3}H]HIAA was almost entirely abolished when tranylcypromine, another MAO inhibitor, was added from 60 to 115 min of superfusion (see below in Table 4).

The time course of the effects of amezinium on the outflow of [^{3}H]noradrenaline and its metabolites is shown in Fig. 1 and further evaluation in Table 2. As little as 1 nM amezinium rapidly and significantly diminished the outflow of total tritium, [^{3}H]DOPEG and [^{3}H]OMDA. With 100 nM amezinium, inhibition of the outflow of [^{3}H]DOPEG was procounced and was accompanied by a marked increase in the outflow of [^{3}H]noradrenaline and [^{3}H]NMN. The effect on the outflow of [^{3}H]DOPEG persisted, whereas the outflow of [^{3}H]noradrenaline and [^{3}H]NMN declined after superfusion with amezinium-free medium had be resumed. Amezinium (1 μM) released so much [^{3}H]noradrenaline that the outflow of total tritium was significantly increased. Interestingly, this high concentration reduced the outflow of [^{3}H]DOPEG less than 100 nM amezinium and did not reduce the outflow of [^{3}H]OMDA at all. The outflow of [^{3}H]DOMA was slightly reduced only by 100 nM amezinium. In the presence of cocaine (which inhibits the high affinity neuronal uptake of noradrenaline), all effects of 100 nM amezinium were abolished or markedly reduced (Fig. 1, Table 2).

Experiments with [^{3}H]dopamine are summarized in Fig. 2 and Table 3. Amezinium (1 nM) caused no change, whereas 10 nM amezinium clearly diminished the outflow of total tritium, [^{3}H]DOPAC and [^{3}H]OMDA. With 100 nM amezinium, these effects were more pronounced, and in addition, the (minimal) outflow of [^{3}H]DOPET was reduced, whereas the outflow of [^{3}H]dopamine was increased. Amezinium (1 μM) released so much [^{3}H]dopamine that the outflow of total tritium was no longer changed. This high concentrations depressed the outflow of [^{3}H]DOPAC slightly less than 0.1 μM amezinium and did not depress the outflow of [^{3}H]OMDA at all. The effects of 1 μM amezinium on the outflow of [^{3}H]DOPAC persisted, whereas the outflow of [^{3}H]dopamine rapidly declined after exposure to amezinium had been discontinued (not shown). In the presence of nomifensine (which inhibits the high affinity neuronal uptake of dopamine), all effects of amezinium 100 nM were abolished or markedly reduced (Fig. 2, Table 3).

Experiments with [^{3}H]serotonin are summarized in Fig. 3 and Table 4. Amezinium (1 nM) caused no change, whereas 10 nM amezinium significantly reduced the outflow of [^{3}H]HIAA. The inhibition was more pronounced with 100 nM amezinium and accompanied by a decrease of total tritium and an increase of [^{3}H]serotonin. The effects rapidly wore

Table 1. Outflow of ^{3}H-compounds from brain slices preincubated with ^{3}H-monamines, and the effect of pargyline and U-0521

Treatment	Outflow of total ^{3}H (pCi/min)	Composition (%)					*n*
		[^{3}H]Noradrenaline	[^{3}H]DOPEG	[^{3}H]DOMA	[^{3}H]NMN	[^{3}H]OMDA	
Control	3366 ± 274	7.0 ± 0.5	33.7 ± 1.6	25.6 ± 3.0	1.0 ± 0.2	32.7 ± 2.1	5
Pargyline	1422 ± 60	60.3 ± 0.5	2.9 ± 0.2	0.5 ± 0.1	21.1 ± 1.5	15.2 ± 2.0	3
U-0521	3413 ± 84	12.1 ± 0.2	40.0 ± 1.4	45.5 ± 1.5	0.1 ± 0.0	2.3 ± 0.2	3
		[^{3}H]Dopamine	[^{3}H]DOPET	[^{3}H]DOPAC	[^{3}H]MTA	[^{3}H]OMDA	
Control	1350 ± 206	7.0 ± 1.1	0.8 ± 0.1	71.1 ± 2.3	2.4 ± 0.2	18.6 ± 1.9	5
Pargyline	657 ± 136	53.5 ± 3.5	0.4 ± 0.1	14.9 ± 1.6	23.0 ± 1.4	8.1 ± 1.9	3
U-0521	905 ± 168	30.2 ± 2.7	0.9 ± 0.1	63.8 ± 3.6	0.4 ± 0.1	4.5 ± 0.9	3
		[^{3}H]Serotonin		[^{3}H]HIAA			
Control	1242 ± 139	11.2 ± 0.4		88.8 ± 0.4			4
Pargyline	2459 ± 505	74.5 ± 1.9		25.5 ± 1.9			3

Occipitocortical slices were preincubated with [^{3}H]noradrenaline (upper panel), or striatal slices were preincubated with [^{3}H]dopamine (middle panel), or occipitocortical slices were preincubated with [^{3}H]serotonin (lower panel). The MAO inhibitor pargyline 75 mg/kg was injected i.p. 21 and 2 hr before sacrifice. The COMT inhibitor U-0521 100 μM was present throughout preincubation and superfusion.

Values refer to the outflow from 55 to 60 min of superfusion.

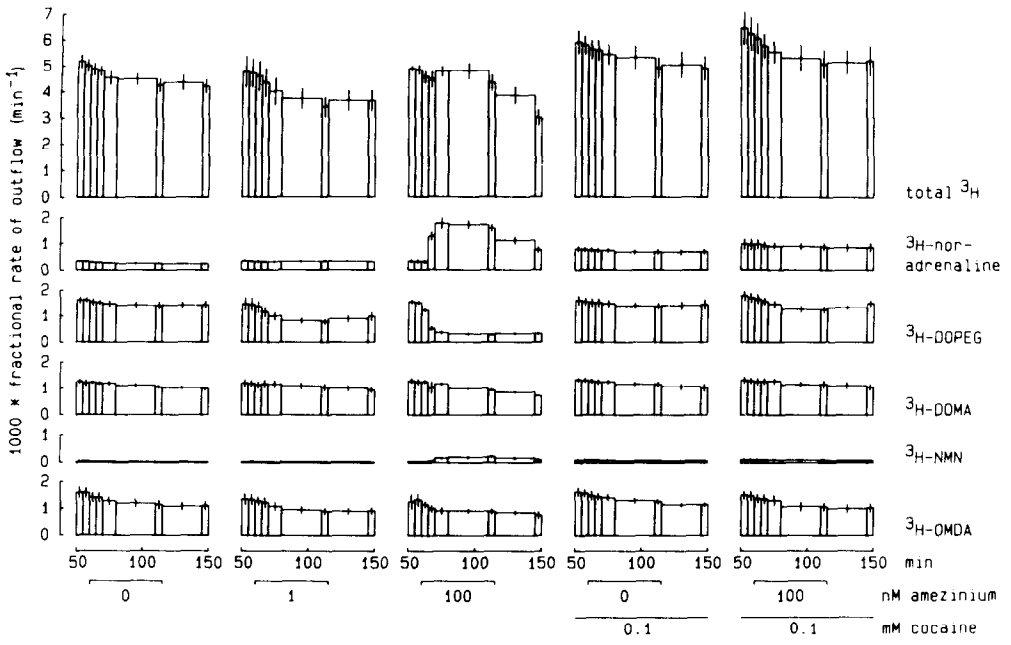

Fig. 1. Effect of amezinium on the outflow of ^{3}H-compounds from occipitocortical slices preincubated with [^{3}H]noradrenaline. After preincubation, the slices were superfused with either drug-free medium (the three left-hand panels) or medium containing 0.1 mM cocaine (the two right-hand panels). Abscissae: minutes of superfusion. Amezinium (0, 1 or 100 nM) was present from 60 to 115 min as indicated by the horizontal bars. Means ± S.E.M. of 3–5 experiments.

off after amezinium had been omitted. Higher concentrations of amezinium released so much [^{3}H]serotonin that the outflow of total tritium was not diminished or actually enhanced; moreover, the outflow of [^{3}H]HIAA was no longer reduced. The outflow of total tritium and [^{3}H]HIAA was significantly diminished, however, after amezinium (1 or 10 μM) had been withdrawn and the outflow of [^{3}H]serotonin had returned to control levels (not shown). Paroxetine (which inhibits the high affinity neuronal uptake of serotonin) abolished all effects of 100 nM amezinium (Fig. 3, Table 4).

Effects of amezinium injected in vivo

In the first series of *in vivo* experiments, rats were decapitated 1 hr after i.p. injection of either tranylcypromine, amezinium or, in parallel control experiments, saline. Formation of [^{3}H]DOPEG upon incubation of occipitocortical cubes for 35 min with [^{3}H]noradrenaline was studied. In control experiments, the formation of [^{3}H]DOPEG amounted to 66.0 ± 11.8 fmole/mg protein. When rats had been treated with tranylcypromine 5 mg/kg, metabolism to [^{3}H]DOPEG was 0.2 ± 0.2% of corresponding controls (n = 3; $P < 0.001$). After injection of 2, 10 and 50 mg/kg amezinium, metabolism to [^{3}H]DOPEG amounted to 95.5 ± 5.1 (n = 7; n.s.), 79.8 ± 15.2 (n = 7; n.s.) and 69.7 ± 10.0% (n = 8; $P < 0.05$) of corresponding controls, respectively. Thus, only the highest dose of amezinium led to a small inhibition of the oxidative deamination of [^{3}H]noradrenaline. The accumulation of [^{3}H]noradrenaline during incubation was not significantly changed by amezinium.

In the second series of *in vivo* experiments, whole brain (minus cerebellum) levels of monoamines were determined 2 hr after i.p. injection of either tranylcypromine, amezinium or saline. In control experiments, concentrations of noradrenaline, dopamine and serotonin were 405 ± 16, 1239 ± 71 and 471 ± 29 ng/g, respectively (n = 7). Tranylcypromine (5 mg/kg) increased these concentrations to 576 ± 57 ($P < 0.05$), 1843 ± 170 ($P < 0.01$) and 864 ± 85 ng/g ($P < 0.01$), respectively (n = 6). In contrast, 50 mg/kg amezinium caused no change (423 ± 10 ng/g noradrenaline, 1210 ± 64 ng/g dopamine and 441 ± 14 ng/g serotonin; n = 7).

DISCUSSION

It is generally accepted that, under our experimental conditions, [^{3}H]noradrenaline, [^{3}H]-dopamine and [^{3}H]serotonin are, at least quite predominantly, taken up into noradrenergic, dopaminergic and serotonergic neurones, respectively [14–16]. The differential effects of amezinium that we observed are in accord with such selective uptake. Marked reduction of MAO- and COMT-metabolites by pargyline (Table 1) or tranylcypromine (Table 4) and U-0521 (Table 1), respectively, confirms the validity of the column chromatographic separation methods.

In occipitocortical slices preincubated with [^{3}H]noradrenaline, as little as 1 nM amezinium significantly reduced the outflow of [^{3}H]DOPEG, [^{3}H]OMDA and, hence, total tritium. Higher concentrations in addition released [^{3}H]noradrenaline, presumably by displacement from the storage ves-

Table 2. Effect of amezinium on the outflow of ^{3}H-compounds from occipitocortical slices preincubated with [^{3}H]noradrenaline

Treatment	Outflow of ^{3}H-compounds (during amezinium/before amezinium)						
	Total ^{3}H	[^{3}H]Noradrenaline	[^{3}H]DOPEG	[^{3}H]DOMA	[^{3}H]NMN	[^{3}H]OMDA	*n*
	0.83 ± 0.01	0.87 ± 0.02	0.87 ± 0.02	0.86 ± 0.02	0.70 ± 0.09	0.82 ± 0.02	5
Amezinium (1 nM)	0.72†	1.00	0.54†	0.87	0.87	0.68†	3
Amezinium (10 nM)	0.65†	2.64†	0.23†	0.78	2.67†	0.50†	3
Amezinium (100 nM)	0.89	5.15†	0.19†	0.74*	5.15†	0.73	3
Amezinium (1000 nM)	1.41†	7.96†	0.28†	0.85	9.78†	0.91	3
Cocaine (0.1 mM)	0.84 ± 0.02	0.88 ± 0.02	0.87 ± 0.01	0.87 ± 0.03	0.71 ± 0.04	0.80 ± 0.02	3
Cocaine (0.1 mM+) amezinium (100 nM)	0.80	0.91	0.72†	0.88	0.92	0.71*	3

The experimental protocol is illustrated in Fig. 1. Values are the ratios between the outflow from 110 to 115 min (the last 5 min sample with amezinium) and the average outflow from 50 to 60 min (the two 5 min samples before amezinium). Standard errors are shown only for controls and experiments with cocaine alone.

Significant differences from corresponding experiments without amezinium: * $P < 0.05$; † $P < 0.01$.

Table 3. Effect of amezinium on the outflow of ^{3}H-compounds from striatal slices preincubated with [^{3}H]dopamine

Treatment	Outflow of ^{3}H-compounds (during amezinium/before amezinium)						
	Total ^{3}H	[^{3}H]Dopamine	[^{3}H]DOPET	[^{3}H]DOPAC	[^{3}H]MTA	[^{3}H]OMDA	*n*
	0.93 ± 0.06	1.36 ± 0.15	1.00 ± 0.11	0.93 ± 0.04	0.55 ± 0.04	0.85 ± 0.05	5
Amezinium (1 nM)	0.96	1.42	0.86	0.96	0.54	0.91	3
Amezinium (10 nM)	0.57†	1.89	1.39	0.45†	0.62	0.60*	3
Amezinium (100 nM)	0.46†	2.04*	0.45*	0.30†	0.74	0.55†	3
Amezinium (1000 nM)	0.86	6.09†	0.30†	0.35†	1.00†	0.70	3
Nomifensine (10 μM)	1.02 ± 0.01	1.21 ± 0.03	1.75 ± 0.52	0.96 ± 0.03	0.84 ± 0.08	0.95 ± 0.03	3
Nominfensine (10 μM) + amezinium (100 nM)	0.91	1.07	1.07	0.75†	0.99	0.96	3

The experimental protocol is illustrated in Fig. 2. Values are the ratios between the outflow from 100 to 115 min (the last 5 min sample with amezinium) and the average outflow from 50 to 60 min (the two 5 min samples before amezinium). Standard errors are shown only for controls and experiments with nomifensine alone.

Significant differences from corresponding experiments without amezinium: *$P < 0.05$; †$P < 0.01$.

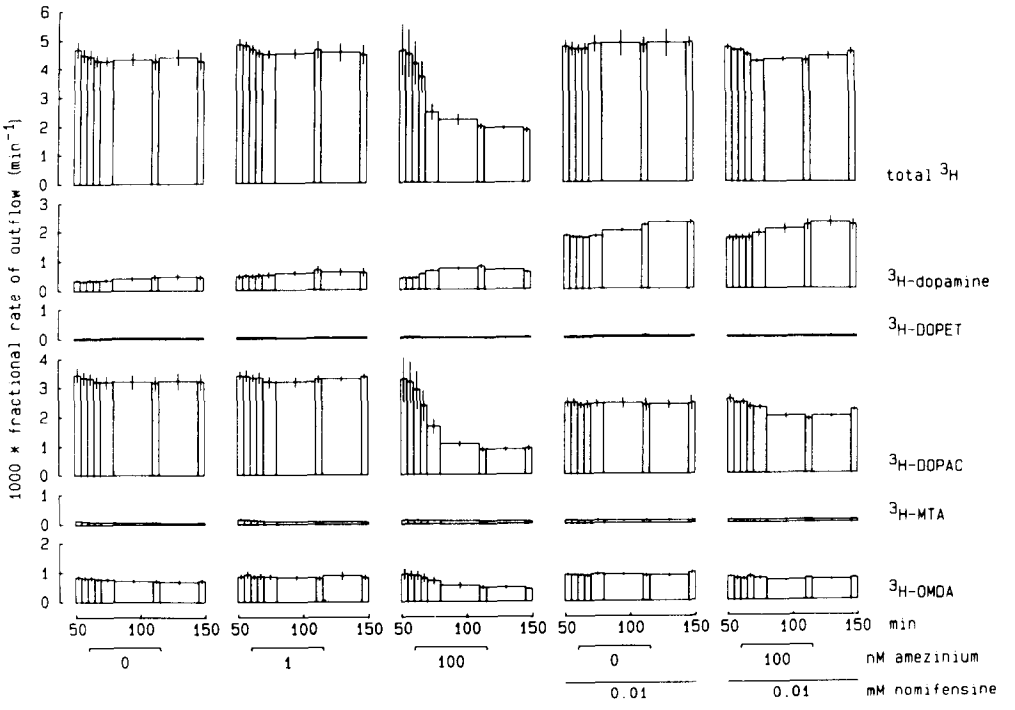

Fig. 2. Effect of amezinium on the outflow of ^{3}H-compounds from striatal slices preincubated with [^{3}H]dopamine. After preincubation, the slices were superfused with either drug-free medium (the three left-hand panels) or medium containing 0.01 mM nomifensine (the two right hand panels). Abscissae: minutes of superfusion. Amezinium (0, 1, or 100 nM) was present from 60 to 115 min as indicated by the horizontal bars. Means ± S.E.M. of 3–5 experiments.

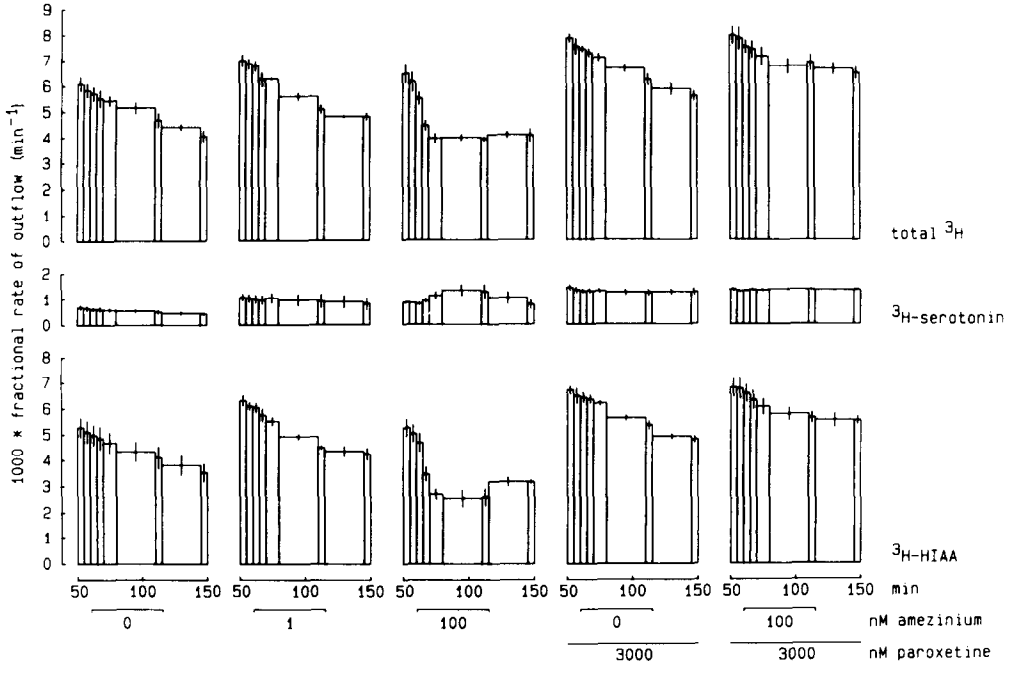

Fig. 3. Effect of amezinium on the outflow of ^{3}H-compounds from occipitocortical slices preincubated with [^{3}H]serotonin. After preincubation, the slices were superfused with either drug-free medium (the three left-hand panels) or medium containing 3000 nM paroxetine (the two right-hand panels). Abscissae: minutes of superfusion. Amezinium (0, 1 or 100 nM) was present from 60 to 115 min as indicated by the horizontal bars. Means ± S.E.M. of 3–4 experiments.

Table 4. Effect of amezinium and tranylcypromine on the outflow of ^{3}H-compounds from occipitocortical slices preincubated with ^{3}H-serotonin

Treatment	Outflow of ^{3}H-compounds (during amezinium/before amezinium)			
	Total ^{3}H	[^{3}H]Serotonin	[^{3}H]HIAA	*n*
	0.78 ± 0.02	0.81 ± 0.06	0.79 ± 0.02	4
Amezinium (1 nM)	0.73	0.72	0.84	3
Amezinium (10 nM)	0.66	0.99	0.61†	3
Amezinium (100 nM)	0.62†	1.47*	0.50†	3
Amezinium (1000 nM)	0.97	3.78†	0.63	3
Amezinium (10,000 nM)	1.85†	8.81†	0.86	3
Tranylcypromine (10 μM)	0.52*	4.13†	0.03†	3
Paroxetine (3 μM)	0.81 ± 0.05	0.89 ± 0.07	0.81 ± 0.04	3
Paroxetine (3 μM) + amezinium (100 nM)	0.87	1.02	0.83	3

The experimental protocol is illustrated in Fig. 3. Tranylcypromine, like amezinium in Fig. 3, was present from 60 to 115 min of superfusion. Values are the ratios between the outflow from 110 to 115 min (the last 5 min sample with amezinium or tranylcypromine) and the average outflow from 50 to 60 min (the two 5 min samples before amezinium or tranylcypromine). Standard errors are shown only for controls and experiments with paroxetine alone.

Significant differences from corresponding experiments without amezinium or tranylcypromine: *$P < 0.05$; †$P < 0.01$.

icles, and increased the outflow of [^{3}H]NMN. The outflow of [^{3}H]DOMA remained largely unchanged. All effects were markedly diminished when amezinium was administered in the presence of cocaine. These results mirror analogous observations on peripheral sympathetically innervated tissues [5]. They indicate that, as in the periphery, amezinium, although a rather weak MAO inhibitor in tissue homogenates [4, 5], becomes a very potent inhibitor of the MAO inside noradrenergic neurones when the cells are left intact, and that this is due to accumulation within the neurones, via the noradrenaline transport mechanism, to high concentrations. Although we did not separate [^{3}H]MOPEG and [^{3}H]VMA in the OMDA fraction, it seems likely that the decrease of [^{3}H]OMDA reflected diminished formation of [^{3}H]MOPEG rather than [^{3}H]VMA [5]. The absence of a marked decrease of [^{3}H]DOMA (and possibly [^{3}H]VMA) is due to the fact that the efflux of these acids does not reflect *de novo* formation but originates from a preformed pool [5, 17].

Amezinium (1 μM) reduced the outflow of [^{3}H]DOPEG and [^{3}H]OMDA less than 0.1 μM amezinium. This was not observed in the periphery, but resembles the findings with cerebral dopamine and serotonin neurones (see below). It seems possible that the large amount of [^{3}H]noradrenaline released by 1 μM amezinium led to appreciable extraneuronal deamination, or that the high concentration of axoplasmic [^{3}H]noradrenaline after displacement from storage vesicles broke through the blockade of MAO by the reversible [4, 5] inhibitor amezinium.

Amezinium acted in a principally similar manner on striatal dopaminergic or cortical serotonergic axons. The formation of MAO metabolites was reduced by relatively low concentrations. Higher concentrations in addition released the transmitter, and the decrease of the MAO metabolites became less pronounced. The release of [^{3}H]dopamine was accompanied by an increase in the outflow of [^{3}H]MTA. All effects of amezinium on the metabolism of [^{3}H]dopamine were markedly conteracted by nomifensine and all effects on the metabolism of [^{3}H]serotonin by paroxetine, indicating again that the respective intraneuronal MAO was the enzyme inhibited and uptake by the carrier mechanisms required for the effect. Analogous *in vitro* effects of amezinium on the metabolism of [^{3}H]dopamine have been obtained in slices of the rabbit caudate nucleus [18].

In spite of these qualitative similarities, there were quantitative differences between the effects of amezinium on noradrenergic, dopaminergic and serotonergic neurones. The effect on the noradrenergic neurones was by far the most prominent. Moreover, amezinium had a somewhat more marked effect on dopaminergic than on serotonergic neurones. For instance, 10 and 100 nM amezinium diminished the outflow of [^{3}H]DOPAC by 52 and 68%, but reduced the outflow of [^{3}H]HIAA by only 23 and 37%, respectively. It seems unlikely that a differential sensivity to amezinium of the MAO enzymes inside the noradrenergic, dopaminergic or serotonergic neurones was responsible for these quantitative differences. Amezinium blocks preferentially MAO A [4], and noradrenaline as well as dopamine and serotonin are mainly deaminated by MAO A in rat brain [19]. The more probable explanation is a difference in the degree of intraneuronal accumulation of amezinium. The uptake of amezinium into various monoaminergic neurones has not been studied directly; but it has been shown that amezinium inhibits the synaptosomal uptake of noradrenaline about 10 times more potently than the uptake of dopamine and the latter at least 10 times more potently than the uptake of serotonin [4], indicating that the affinities for the respective carrier mechanisms decline in the order noradrenaline > dopamine > serotonin.

As mentioned in the Introduction, 0.01 mg/kg amezinium, injected i.v. into rabbits, inhibited the oxidative deamination of noradrenaline in the postmortally removed and subsequently perfused heart [5]. In contrast, 50 mg/kg amezinium, injected i.p. into rats, were necessary in the present experiments for some inhibition of the oxidative deamination of noradrenaline in cubes prepared from the postmortally removed brain. This discrepancy is probably not due to diffusion of amezinium from the cubes into the assay mixture and, hence, dilution of the inhibitor; although amezinium interacts with MAO reversibly [4, 5], it is avidly retained at its site of action, i.e. in the noradrenergic neurones, by vesicular storage and re-uptake after release [7] (this retention explains the prolonged decrease of the formation of DOPEG and DOPAC after superfusion of brain slices with amezinium had been discontinued; see Figs. 1 and 2). More extensive hepatic degradation of i.p. injected amezinium (rat brain) than i.v. injected amezinium (rabbit heart) may contribute to the lower potency in the present experiments. However, the main reason is probably an only minor passage of amezinium across the blood–brain barrier [20]. Very high doses of amezinium were also required to elicit central effects in mice [8].

The inhibition of cerebral MAO described in this article, and inhibition of the re-uptake of monoamines, might explain the indications of an antidepressant effect found in man by Saletu *et al.* [9]. However, these authors used low doses (10 or 20 mg total dose); if we can extrapolate from the present results to humans, an interference of such low doses with cerebral monoamine metabolism would not be expected. It seems possible that in the study on humans a primary peripheral action of amezinium such as an increase in blood pressure contributed to the observed changes in brain activity. Nevertheless, a compound sharing with amezinium its neurochemical effects, but with easier access to the brain, might be interesting neuropharmacologically, for instance, in depression or Parkinson's disease.

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