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Decreased distribution of oxotremorine to brain after pharmacological blockade of its peripheral acetylcholine-like effects

BO KARLÉN,* LIL TRÄSKMAN† AND FOLKE SJÖQVIST†

**Department of Organic Chemistry, Faculty of Pharmacy, Box* 6804, 113 86 *Stockholm, and †Department of Pharmacology (Division of Clinical Pharmacology), Karolinska Institutet, Stockholm* 60, *Sweden.*

The distribution of [^{3}H]oxotremorine, after intravenous injection, to brain and tissues of rats and mice has been studied. In rats, oxotremorine (0·3 mg/kg) rapidly reached the brain with a peak concentration of 1200 ng/g at 1 min. Blockade of the peripheral muscarinic effects of oxotremorine with amitriptyline methyl iodide or atropine methyl nitrate (25 mg/kg, s.c.) markedly decreased the brain content of oxotremorine (peak concentration 400 ng/g), probably as a result of its more rapid distribution to peripheral tissues and an increased volume of distribution. The ratio of drug distribution between plasma and brain is unaffected by pretreatment with anticholinoceptive drugs. Also, the total body concentrations of oxotremorine are similar in pretreated rats and controls. A small dose of oxotremorine (10 μg/kg) is distributed similarly in pretreated rats (amitriptyline methyl iodide, 25 mg/kg, s.c.) and in controls. In mice there was a linear relation between dose, up to 180 μg/kg, and brain concentration of oxotremorine. At higher doses, relatively more drug reached the brain. Oxotremorine induced a marked fall in blood pressure in the unanaesthetized rat that was reduced by amitriptyline methyl iodide and reversed by atropine methyl nitrate. It seems likely that differences in blood flow between pretreated rats and controls are responsible for the differences in the distribution of the drug.

Tremorine (1,4-dipyrrolidino-2-butyne) and oxotremorine [1(2-oxopyrrolidino)-4-pyrrolidino-2-butyne] induce in laboratory animals a syndrome resembling the symptoms of Parkinson's disease, i.e. ataxia, tremor and rigidity (Everett, Blockus & Shepperd, 1956), that can be blocked by antiparkinsonian drugs. Tremorine and oxotremorine, therefore, have been used widely in the screening of compounds with potential activity in this disease (Jenden, 1967). Since tremorine owes its effect to its biotransformation to oxotremorine (Cho, Haslett & Jenden, 1961), and various blockers of tremorine metabolism interfere in the screening procedure (Sjöqvist, Hammer & others, 1968), oxotremorine has replaced tremorine in this test. The assumption is made that the intensity of induced tremor depends on the concentration of oxotremorine in the brain and that the antagonists interfere with the drug's action solely at central receptor sites.

Oxotremorine also produces hypothermia, and drugs that counteract this effect may enhance its disappearance from the brain (Hammer, Karlén & Sjöqvist, 1968a). Such drugs decrease the intensity and duration of tremor partly by lowering the concentration of oxotremorine at receptor sites in brain (loc. cit.).

We report a marked impairment of the distribution of the drug to the brain after its peripheral acetylcholine-like effects had been blocked. This represents a new type

of drug interaction. The investigation was precipitated by the finding that the hypothermic effect of oxotremorine in mice was reduced by amitriptyline methyl iodide although this compound should not pass the blood brain barrier readily.

MATERIAL AND METHODS

Drugs and animals

[^{3}H]Oxotremorine was prepared and checked for radiochemical purity (Karlén & Telč, 1966; Hammer, Karlén & Sjöqvist, 1968b) and diluted with unlabelled oxotremorine oxalate to yield a specific activity (10 mCi; mmol) which was convenient for analysis.

Male Sprague Dawley rats, 180–200 g, or male Swiss albino mice (N.M.R.I., Bethesda), 18–22 g, were used. Oxotremorine (0·3 mg/kg as base or as otherwise stated) was administered into the tail vein in a volume of 1 or 5 ml/kg (rat or mouse respectively). Thirty min before the administration of drug, rats or mice were injected subcutaneously with either saline, or atropine methyl nitrate (25 mg/kg), or amitriptyline methyl iodide (25, 12·5 or 6·25 mg/kg). Rats were also pretreated with *N*(5-pyrrolidino-3-pentynyl) succinimide citrate (BL 14) (10 mg/kg as base, i.p.) in volumes of 1 ml/kg, 15 min before oxotremorine.

Determination of [^{3}H]oxotremorine

Rats were killed with ether, decapitated and blood collected. Plasma and various tissues were removed and analysed.

Plasma. Plasma (1 ml) was made alkaline with NaOH (1 ml; 0·5N) and extracted with toluene (6 ml) containing 1·5% isoamylalcohol. After centrifugation, 4 ml of the organic phase was mixed with toluene (10 ml) mix (PPO/POPOP) and counted by liquid scintillation. The extraction of oxotremorine was not affected by the presence of amitriptyline methyl iodide or atropine methyl nitrate.

Brain, liver, kidney and lung. The tissues were dissected out, weighed and homogenized in an Ultra-turrax homogenizer in seven parts of ice-cold water and a sample of the homogenate (2 ml) was made alkaline, extracted, and counted as described above.

Carcass. This was homogenized in a Waring blendor with four parts of ice-cold water. A sample of the homogenate (2 ml), filtered through gauze, was made alkaline, extracted and counted as described above.

Red cells. To red cells (1 ml) was added NaOH (1 ml; 0·5N). The mixture was frozen and the resulting thick emulsion was diluted with water (2 ml), extracted and and counted.

Bladder plus urine. The ureters were tied off and the bladder containing the urine prepared free and weighed. The bladder was homogenized with an Ultra-turrax homogenizer with ten parts of water and a 2 ml aliquot was analysed as above.

Internal standards. To an aliquot of plasma or tissue homogenate from untreated animals was added a known amount of [^{3}H]oxotremorine in a volume of 10 μl. The standards and samples of unknown concentration were analysed together.

Specificity of the extraction procedure. The extraction procedure is specific for oxotremorine (Hammer & others, 1968a).

Recording of body temperature. The rectal temperature of the mice (rats show no hypothermia after the drug) was recorded with an electrothermometer at intervals of 30 min throughout the experiments, which were made at 20–22°.

Recording of blood pressure. Rats were anaesthetized with ether. A polyethylene tube was inserted in a carotid artery and connected to a Statham pressure transducer model P 23 DC. The rats were allowed to recover from anaesthesia for about 1 h and were then restrained in a suitably sized plastic tube. Oxotremorine was then injected in the tail vein and the blood pressure recorded for about a minute, whereupon the rat was removed from the restrained position and placed free on the laboratory bench.

Surgical procedures

The distribution of oxotremorine was studied after bilateral nephrectomy performed transabdominally in a few rats anaesthetized either with sodium pentobarbitone (20–40 mg/kg, i.p.) or ether. After ligating the blood vessels and suturing the abdomen the rats were allowed to recover the righting reflex before oxotremorine was administered.

RESULTS

Effect of amitriptyline methyl iodide on the distribution of tremorine in rats

Rats were pretreated with saline, then given the drug (0·3 mg base/kg, i.v.) and killed at various times thereafter. The drug disappeared from plasma polyphasically. A peak brain concentration of 926 ng/g was reached within 1 min, after which the concentration declined monophasically (Fig. 1). When rats were pretreated with amitriptyline methyl iodide (25 mg/kg, s.c.) 30 min before the injection of the drug, 1 min after the injection the plasma concentration in the pretreated rats was 300 ng/ml (compared with 1200 ng/ml in the controls), and the peak brain concentration of the pretreated animals was only 380 ng/g which was reached 2 min after the injection.

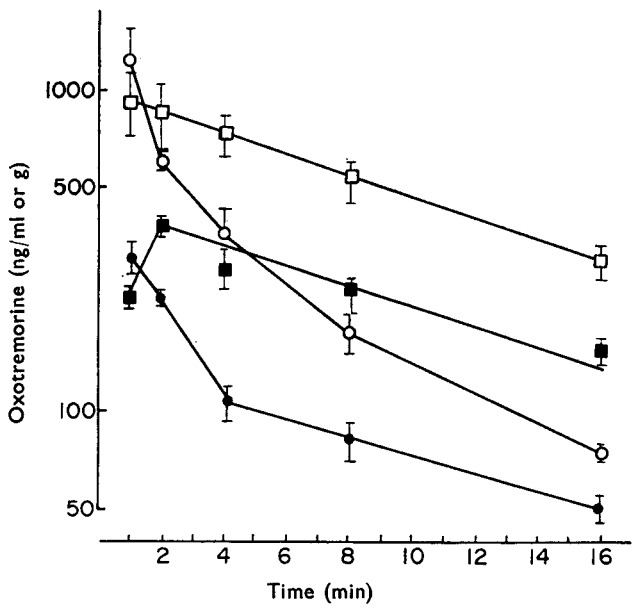

FIG. 1. Distribution of oxotremorine (0·3 mg/kg, i.v.) in rats after pretreatment with amitriptyline methyl iodide (25 mg/kg, s.c). Data are given as means ± s.d. (n = 3). ○—○ Plasma and □—□ brain controls. ●—● Plasma and ■—■ brain amitriptyline methyl iodide.

The ratio between the concentrations of drug in plasma and brain were similar in the absence and presence of amitriptyline.

The control rats developed tremor and the usual peripheral symptoms after the drug while pretreated rats showed tremor but no peripheral symptoms.

Distribution of oxotremorine after different pretreatment doses of amitriptyline methyl iodide

Rats were pretreated with amitriptyline methyl iodide (6·25, 12·5 and 25 mg/kg) and then given oxotremorine (0·3 mg base/kg, i.v.). At all doses of the amitriptyline salt, plasma and brain concentrations of oxotremorine (after 8 min) were significantly lower (about 85 ng/ml and 250 ng/g, respectively regardless of the dose of amitriptyline) than in the controls (181 ± 4 ng/ml and 458 ± 47 ng/g respectively); data are means ± s.d. of three animals. All pretreated rats were protected from the peripheral effects of the drug.

Distribution of a small dose of oxotremorine

To test whether the distribution of oxotremorine could be related to its pharmacological effects, the distribution and elimination of a small dose (10 μg/kg, i.v.), insufficient to produce any visible symptoms, was studied. Brain concentrations at 4 and 16 min were almost identical in control rats (9·8 ± 0·9 and 6·6 ± 0·4 ng/g, respectively) and in rats pretreated with amitriptyline methyl iodide (25 mg/kg, s.c., 9·8 ± 1·9 and 6·5 ± 0·9 ng/g, respectively). Plasma levels in the control rats (3·8 ± 0·6 and 1·5 ± 0·2 ng/g, respectively) were somewhat lower than in pretreated rats (4·3 ± 0·7 and 2·0 ± 0·5 ng/g, respectively). Data are expressed as means ± s.d. of three or four animals.

Distribution of oxotremorine in tissues

Rats were pretreated with amitriptyline methyl iodide (25 mg/kg, s.c.) or saline followed by oxotremorine (0·3 mg/kg, i.v.) 30 min later and killed after 8 min. The concentration of drug was lower in plasma, brain and lung of pretreated animals compared to controls (Tables 1 and 2), whereas the concentrations in kidney, liver and carcass did not differ significantly between the groups (Table 1). The ratio between the concentration of oxotremorine in carcass and plasma was doubled in the pretreated animals. The urine from bladders of pretreated rats was greater in volume and had about 30 times higher concentration of the drug compared to controls.

Table 1. *Concentrations of oxotremorine (OTMN) in different tissues after pretreatment with amitriptyline methyl iodide in rats.*

		Concn of OTMN (ng/ml or g ± s.d.)						
Pretreatment	Dose (mg/kg, s.c.)	Plasma	Brain	Lung	Kidney	Liver	Urine + bladder (pooled)	Carcass
Controls ..	Saline	177 ± 10	583 ± 74	312 ± 69	1018 ± 361*	56 ± 17*	220 (0·76 g)	206 ± 14*
Amitriptyline methyl iodide	25	94 ± 23	319 ± 66	210 ± 23	990 ± 347*	40 ± 3*	6315 (2·07 g)	182 ± 23*

Rats were pretreated 30 min before the administration of OTMN (0·3 mg/kg, i.v.) and killed 8 min thereafter. The data are expressed as means ± s.d. from three animals.
* N.S.

Table 2. *Plasma, red cell and brain concentrations of oxotremorine (OTMN).*

Pretreatment	Dose (mg/kg, s.c.)	Time (min)	Concn of OTMN: Plasma (ng/ml)	Red cells (ng/g)	Brain (ng/g)
Control	Saline	1	887 (2)	765 (2)	1012 (2)
,,	,,	8	155 ± 27	129 ± 21	637 ± 292
Amitriptyline methyl iodide ..	25	1	389 ± 15	340 ± 7	392 ± 43
,,	,,	8	105 ± 7	92 ± 9	297 ± 91

Rats were pretreated 30 min before the administration of OTMN (0·3 mg/kg, i.v.).
The data are expressed as means ±s.d. of three animals.

Red cells as well as plasma and brain were analysed for oxotremorine in pretreated (amitriptyline methyl iodide, 25 mg/kg, s.c.) and control rats at 1 and 8 min after the drug (Table 2). Its concentration in the red cells was lower than in plasma both in control and pretreated rats at each time. There was no difference between the two groups in the ratio between the concentration of drug in plasma and red cells.

Distribution of oxotremorine in nephrectomized rats

To exclude the possibility that differences in renal clearance of oxotremorine between pretreated and control rats could explain the phenomena observed, the kidneys of one group of rats were removed under anaesthesia, while two other groups of rats were sham-operated. The nephrectomized and one of the sham-operated groups were pretreated (30 min) with amitriptyline methyl iodide (6·25 mg/kg, s.c.), while the third group was given saline. Oxotremorine (0·3 mg/kg, i.v.) was then given and the animals killed 2 min later. The results in Table 3 show no marked difference between the pretreated nephrectomized and sham-operated rats, but both groups were significantly different from the controls. Thus the brain and plasma concentrations of drug were significantly higher in the control rats. The concentrations of drug in the carcass were the same in all three groups.

Table 3. *Plasma and brain concentrations of oxotremorine (OTMN) in nephrectomized rats.*

	Pretreatment	Dose (mg/kg, s.c.)	Experiment No.*	Concn of OTMN: Plasma (ng/ml)	Brain (ng/g)	Carcass (ng/g)
Nephrectomized	Amitriptyline methyl iodide	6·25	I	281 (2)	633 (2)	208 (2)
	,, ,,	,,	II	460 (2)	763 (2)	—
Sham-operated	Amitriptyline methyl iodide	6·25	I	275 (2)	676 (2)	234 (2)
	,, ,,	,,	II	394 ± 12	671 ± 10	—
Sham-operated	Controls	Saline	I	491 (2)	991 (2)	210 (2)
	,,	,,	II	670 ± 39	1187 ± 210	—

Rats were pretreated 30 min before the administration of OTMN (0·3 mg/kg, i.v.) and killed 2 min later. The data are expressed as means of two animals or as means ± s.d. of three animals.
* In exp. I rats were anaesthetized with sodium pentobarbitone, 20–40 mg/kg, i.p.
In exp. II rats were anaesthetized with ether.

Brain concentrations of oxotremorine in mice

Parts of the above experiments were repeated in mice using the brain only. The data indicate a similar difference in the distribution of oxotremorine as in rats between controls and pretreated (amitriptyline methyl iodide, 25 mg/kg, s.c.) mice (controls 407 ± 23, 378 ± 20; pretreated animals 230 ± 3, 267 ± 13 μg drug/g brain) at 2 and 8 min respectively.

The brain concentration of oxotremorine was also analysed after oxotremorine at doses of 60, 180, 300 and 360 μg/kg; the concentrations of drug in the brain at 2 min were 60 ± 9, 182 ± 20, 407 ± 23 and 501 ± 49 ng/g, respectively (means ± s.d. of five animals).

Up to doses of 180 μg/kg of oxotremorine there was a linear relation between the dose and concentration in the brain. However, at higher doses relatively more drug was distributed to the brain.

Hypothermic effect of oxotremorine in mice

The hypothermic effect of oxotremorine in mice was partly blocked by amitriptyline methyl iodide.

Distribution of oxotremorine after pretreatment with atropine methyl nitrate and N(5-*pyrrolidino*-3-*pentynyl*)*succinimide* (*BL* 14)

Rats were pretreated with either saline or atropine methyl nitrate (25 mg/kg, s.c.) 30 min before receiving oxotremorine (0·3 mg/kg, i.v.), and were killed at various times thereafter. Plasma and brain concentrations of drug were essentially the same as those obtained with amitriptyline methyl iodide. The animals showed fully developed tremor but no peripheral effects of oxotremorine.

The tertiary amine, BL 14, which antagonizes both central and peripheral symptoms of oxotremorine (Karlén, Lindeke & others, 1970), was also used for pretreatment (10 mg/kg, i.p., 15 min before oxotremorine, 0·3 mg/kg, i.v.). Results were similar to those obtained with the two other anti-acetylcholine drugs. The pretreated rats showed no salivation or lacrimation and no (or very slight) tremor after oxotremorine.

Blockade of blood pressure lowering effect of oxotremorine in the unanaesthetized rat with amitriptyline methyl iodide and atropine methyl nitrate

Oxotremorine (0·3 mg/kg, i.v.) evoked a dramatic fall (−105 ± 13·7 mm Hg) in the arterial blood pressure of the unanaesthetized rat. Pretreatment with atropine methyl nitrate in the dose used in the pharmacokinetic experiments (25 mg/kg, s.c.) reversed this effect and in contrast a rise (+82 ± 7·2 mm Hg) in blood pressure was induced by oxotremorine (Walker & Weetman, 1970). Pretreatment with amitriptyline methyl iodide (25 mg/kg, s.c.) partially reduced (−68 ± 6·5 mm Hg) the hypotensive effect of oxotremorine (means ± s.d. of 3–7 animals).

DISCUSSION

Plasma and brain concentrations of oxotremorine after intravenous injection were much lower in rats and mice in which the peripheral anti-acetylcholine effects of the drug were blocked, than in control animals. In contrast, the distribution of a small, pharmacologically ineffective dose of oxotremorine is similar in control rats and in rats pretreated with amitriptyline methyl iodide. These findings indicate that the pharmacological effects of oxotremorine greatly influence its own distribution, a new

example of dose-dependent pharmacokinetics (cf. Levy, 1968). Data in mice show that relatively more of the drug is distributed to the brain when the dose is increased above a certain level. Oxotremorine has a profound hypotensive effect in the unanaesthetized rat. This will result in marked changes in the blood-flow to different tissues.

After pretreatment with anti-acetylcholine drugs it therefore seems likely that the distribution of oxotremorine will be different compared to that in animals with the fully developed oxotremorine-syndrome.

Our results indicate that oxotremorine is much more rapidly distributed from plasma to tissues when its peripheral acetylcholine-like and hypotensive effects have been blocked fully or partly. The kinetic data suggest that the volume of distribution of the drug is increased in rats pretreated with anti-acetylcholine drugs. Already after 1 min the plasma level of oxotremorine is one third of that in the control rats (Fig. 1).

The low plasma concentrations of oxotremorine in pretreated animals are responsible for the lower brain concentrations compared to controls. There is no evidence for an effect of the anti-acetylcholine drugs on the diffusion of oxotremorine from plasma to brain. The small increase in the renal excretion of the drug after pretreatment with amitriptyline methyl iodide cannot account for the much lower plasma and brain concentrations of the drug in pretreated animals compared to those in controls. This is further shown in the experiments with nephrectomized rats. Also, the oxotremorine-level in the carcass was similar in all experimental groups.

The pharmacological significance of our findings is obvious since lowered brain concentrations of the drug in the pretreated animals will result in less marked central symptoms. This may therefore be unrelated to an antagonism between oxotremorine and the pretreatment drug at receptor sites in brain. In fact, amitriptyline methyl iodide although a quaternary ammonium base thought not to pass the blood-brain barrier, reduced the hypothermia caused by oxotremorine. Our experiments illustrate the fallacies with drug screening procedures utilizing drug-induced "models" of human diseases. It is important in such procedures to measure the concentrations of the interacting drugs in body fluids or tissues, a fact which is usually disregarded.

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