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EFFECT OF OXOTREMORINE ON ENDOGENOUS ACETYLCHOLINE AND ON UPTAKE AND BIOTRANSFORMATION OF RADIOACTIVE CHOLINE IN DISCRETE REGIONS OF MOUSE BRAIN *IN VIVO*

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Abstract—Oxotremorine (1 mg/kg) was injected intraperitoneally 15 min before an intravenous dose of 15 nmoles of tritium-labelled choline ([^{3}H]choline). The animals were sacrificed 1 or 5 min later by dislocation of the spine. Hypothermia was prevented by a heating lamp. The brains were rapidly dissected into six well-defined regions (cerebellum, medulla oblongata, midbrain, striatum, hippocampus and cortex). Endogenous acetylcholine was significantly ($P < 0.01$) increased in the striatum (+87%), hippocampus (+49%) and cortex (+115%) but unchanged in the cerebellum, medulla oblongata and midbrain. Pretreatment with methylatropine (5 mg/kg) and with atropine (5 mg/kg) partly counteracted the increase of endogenous acetylcholine in the cortex, and atropine also had the same effect in the striatum. The biosynthesis of [^{3}H]acetylcholine at 1 and 5 min was decreased in all regions except the striatum. This was prevented by pretreatment with atropine (5 mg/kg); methylatropine (5 mg/kg) was considerably less effective. In the striatum the formation of [^{3}H]acetylcholine was increased (+74–101%) after administration of oxotremorine. The increase was not antagonized by pretreatment with atropine (5 mg/kg) or methylatropine (5 mg/kg). Oxotremorine produced a marked decrease in the specific radioactivities of acetylcholine in the hippocampus and cortex but not in the striatum.

Oxotremorine, a muscarinic agonist with pronounced and characteristic pharmacodynamic effects on the CNS, increases the level of endogenous acetylcholine [1, 2] and decreases the turnover of acetylcholine [3, 4] in whole brain. Studies on the effect of oxotremorine on endogenous acetylcholine in different brain regions have revealed selective increases in the cortex and caudate nucleus in the rat [5] and in the diencephalon and upper part of the midbrain in the cat [6]. The present study deals with the effect of oxotremorine on endogenous acetylcholine and on the uptake and rate of biotransformation of tracer doses of tritium-labelled choline ([^{3}H]choline) in discrete regions of mouse brain *in vivo*.

MATERIALS AND METHODS

General. Female mice (NMRI strain) weighing 18–22 g were used. At least 6 days before the experiments the animals were placed in a silent room with constant diurnal lighting (artificial) of 12 hr light and 12 hr dark. The light was switched on at 6.00 a.m. Food and water were supplied *ad lib.* The experiments were always performed between 10.00 and 11.30 a.m.

Fifteen nmoles of tritium-labelled choline [*methyl*-^{3}H]choline, 16·5 Ci/m-mole, purchased from the Radiochemical Centre, Amersham, England) were dissolved in 100 μl of 0·9% saline and injected into a tail vein (in 2–3 sec). One, 5 or 10 min later the animals were sacrificed by dislocation of the spine. When the brain had been removed from the skull it was placed on an ice-cold glass plate and rapidly dissected into cerebellum, medulla oblongata + pons, midbrain (including hypothalamus), striatum, hippocampus and cortex, essentially according to the method described by Glowinski and Iversen [7]. The brain tissues were weighed and then homogenized (10 strokes), and extracted with 7% ice-cold trichloroacetic acid (TCA). The time taken from sacrifice to inactivation of enzymes with TCA was about 7 min. The homogenates were kept in a cold room (+5°) for 30 min and then centrifuged at 4000 rev/min for 10 min (for cortex 6000 rev/min, 15 min). The pellets were resuspended with TCA and centrifuged. Excess of TCA was removed from the combined supernatants by extraction with portions of ether until the pH was 4. The extracts were freeze-dried and dissolved in 100 μl of glass-redistilled water.

Separation and determination of radioactivity. Twenty μl of the solution were used for assay of the total radioactivity (^{3}H-tot) and the same volume was submitted to high voltage electrophoresis at pH 4·8 in a buffer containing pyridine, acetic acid, acetone and water (8:8:30:154 by volume). The main metabolites, [^{3}H]phosphorylcholine, [^{3}H]acetylcholine and [^{3}H]choline were localized by scanning the paper in a radiochromatograph (Packard). The paper strips were eluted with water and the radioactivity was measured by liquid scintillation in a Nuclear Chicago Isocap 300 scintillation counter. For further details see Nordberg and Sundwall [8, 9].

Assay of endogenous acetylcholine. Endogenous acetylcholine was measured by bioassay on the dorsal muscle of leech suspended in a microbath [10, 11]. The sensitivity limit of the procedure is 0·5 pmoles in 50 μl bathing fluid. Insignificant contractions were obtained with extracts treated with alkali (pH 11–12) for 30 min or when *d*-tubocurarine was added to the

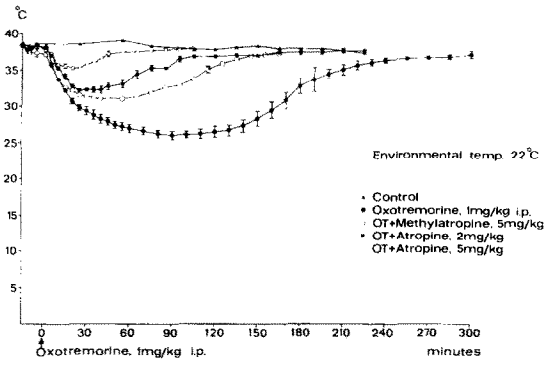

Fig. 1. Effect of atropine and methylatropine on hypothermia produced by oxotremorine (OT), 1 mg/kg (rectal temperature). Three groups of animals were pretreated with methylatropine (5 mg/kg) or atropine (2 or 5 mg/kg) 15 min before injection of oxotremorine. Controls received saline. Each point represents the mean value of 3–4 measurements. Vertical bars indicate S.E.

bath. Addition of known amounts of acetylcholine to the samples revealed no inhibition or potentiation.

Experiments with oxotremorine. Oxotremorine oxalate (mol. wt 341) was dissolved in 0·9% saline, and 1 mg/kg (corresponding to 0·6 mg/kg free base) in 100 μl saline was injected intraperitoneally (i.p.) 15 min prior to an intravenous (i.v.) injection of [^{3}H]choline. The animals were sacrificed 16 or 20 min after treatment with oxotremorine (1 or 5 min after [^{3}H]choline). Endogenous acetylcholine was determined in animals sacrificed 20 min after injection of oxotremorine. To protect the animals from heat loss they were warmed by an infrared lamp. The rectal temperature was measured before and repeatedly during the experiment and kept at 38° by adjusting the lamp. The animals were carefully observed during the experiments and certain symptoms such as tremor, salivation and rigidity were rated and recorded.

Pretreatment with atropine or methylatropine. Atropine sulphate and atropine methylnitrate were dissolved in 0·9% saline. An i.p. dose of 2 or 5 mg/kg atropine sulphate (corresponding to 1·7 or 4·2 mg/kg free base) or 5 mg/kg atropine methylnitrate (4·2 mg/kg free base) was given 15 min before the injection of oxotremorine.

Studies of hypothermic effect. The rectal temperature was followed for 4–5 hr without any compensation for heat loss in five groups of animals (oxotremorine alone and oxotremorine preceded 15 min earlier by 2 or 5 mg/kg atropine sulphate or 5 mg/kg atropine methylnitrate). The fifth group constituted the controls and received 100 μl of 0·9% saline. The rectal temperature was measured with an electrothermometer (Farad Electronics, Stockholm). The experiments were run at a room temperature of 22°.

Studies with [^{3}H]dextran. Five nmoles of tritium-labelled dextran ([^{3}H]dextran, 26 mCi/g purchased from the Radiochemical Centre, Amersham, England) dissolved in 100 μl 0·9% saline were injected into a tail vein 6 min after i.p. injection of 1 mg/kg oxotremorine. Heat loss was compensated. Ten min after injection of [^{3}H]dextran the animals were killed by dislocation of the spine. Blood samples were taken shortly before death. The brain was dissected into six discrete regions and weighed. The brain tissues and blood samples were oxidized in a sample oxidizer (Packard) and the amount of radioactivity was measured by liquid scintillation.

RESULTS

Effect of atropine and methylatropine on the hypothermia produced by oxotremorine. The hypothermic effect of oxotremorine oxalate (1 mg/kg, i.p.) is shown in Fig. 1. During the first 20 min the rectal temperature decreased almost linearly from 38·5 ± 0·13 to 30·7 ± 0·39°. It continued to drop and a minimum level was reached at 90 min (26·1 ± 0·54°). The temperature then slowly increased and was almost normal at 5 hr. The effect was reduced to some extent by pretreatment with 5 mg/kg of methylatropine or 2 mg/kg of atropine. Atropine in a dose of 5 mg/kg had a stronger antagonistic effect and resulted in a maximum drop of 3 deg and a return to normal after about 1·5 hr. In animals pretreated with methylatropine and with the lower dose of atropine the maximum drop in temperature was almost the same (about 6–7°).

Effects of atropine and methylatropine on tremor, rigidity, salivation, lachrymation, and mydriasis produced by oxotremorine. In these experiments heat loss was prevented by adjustment of an infrared lamp. The findings are summarized in Table 1. Two to 3 min after the intraperitoneal injection of oxotremorine the animals began to show tremor, salivation and lachrymation. Their hind legs were stiff and outstretched, impeding their movements. The tail was also rigid and the pupils were enlarged. Methylatropine (5 mg/kg) prevented the salivation and lachrymation but not the tremor, the rigidity of the tail or the effects on the hind legs. The pupils were 2–3 times larger than

Table 1. Effects of atropine and methylatropine on the symptoms produced by oxotremorine oxalate (1 mg/kg i.p.) in mice compensated for heat loss

Symptoms	Oxotremorine	Methylatropine (5 mg/kg) + oxotremorine	Atropine (2 mg/kg) + oxotremorine	Atropine (5 mg/kg) + oxotremorine
Tremor	+++	+++	(+)	0
Salivation	+++	0	0	0
Lachrymation	++	0	0	0
Mydriasis	+	+++	+++	+++
Rigidity of tail	+++	+++	0	0
Rigid outstretched hindlegs	+++	+++	++	+

0 no effect; + threshold effect; ++ moderate effect; +++ marked effect.

Table 2. Effect of oxotremorine (1 mg/kg, i.p.) on endogenous acetylcholine (nmoles/g) in discrete regions of mouse brain *in vivo*

Brain region	Control	Oxotremorine (1 mg/kg, i.p.)	Methylatropine (5 mg/kg, i.p.) + oxotremorine	Atropine (5 mg/kg, i.p.) + oxotremorine
Cerebellum	4·3 ± 0·30 (7)	4·1 ± 0·22 (3)		
Medulla oblongata	21·6 ± 0·65 (8)	23·4 ± 1·09 (3)		
Midbrain	20·7 ± 1·47 (8)	22·4 ± 2·04 (3)		
Striatum	40·1 ± 1·71 (8)	75·0 ± 3·50 (3)†	75·5 ± 2·16 (3)†	64·1 (60·1; 68·1)
Hippocampus	17·0 ± 0·90 (8)	25·4 ± 1·67 (3)†		
Cortex	13·3 ± 0·95 (7)	28·6 ± 1·14 (3)†	19·6 ± 0·73 (3)*	20·0 (19·7; 20·2)
Whole brain	15·4 ± 0·54 (7)	25·2 ± 0·39 (3)†		

M$\bar{v}$ ± S.E.; (n) = number of experiments; * $P < 0.01$, † $P < 0.001$ compared with control.

in the animals treated with oxotremorine alone. Atropine (2 or 5 mg/kg) had the same effects and in addition antagonized the tremorigenic action of oxotremorine. However, the effect on the hind legs persisted slightly even after administration of 5 mg/kg of atropine.

Effect of oxotremorine on endogenous acetylcholine in discrete brain regions. Table 2 shows the endogenous amount of acetylcholine in different brain regions 20 min after i.p. injection of 1 mg/kg of oxotremorine. The animals were compensated for heat loss. Endogenous acetylcholine was significantly ($P < 0.01$) increased in the striatum (+87%), hippocampus (+49%) and cortex (+115%) but was unchanged in the midbrain, medulla oblongata and cerebellum. In animals pretreated with 5 mg/kg of methylatropine or 5 mg/kg of atropine, oxotremorine still increased the content of endogenous acetylcholine in the striatum and cortex, which were the only regions studied in this respect. However, both drugs partially antagonized the effect of oxotremorine in the cortex (about 50%). Methylatropine had no effect on the acetylcholine increase in the striatum.

Effect of oxotremorine on uptake, distribution and biotransformation of [^{3}H]choline in discrete brain regions. Fifteen min after treatment with oxotremorine (1mg/kg) the mice were injected i.v. with [^{3}H]choline and were sacrificed by dislocation of the spine 1 or 5 min later. The animals were compensated for heat loss. The results are presented in Figs. 2–5. The uptake of radioactivity (^{3}H-tot) (Fig. 2) was increased in all brain regions at both 1 and 5 min (16–65%) as compared with control animals. Figure 3 shows

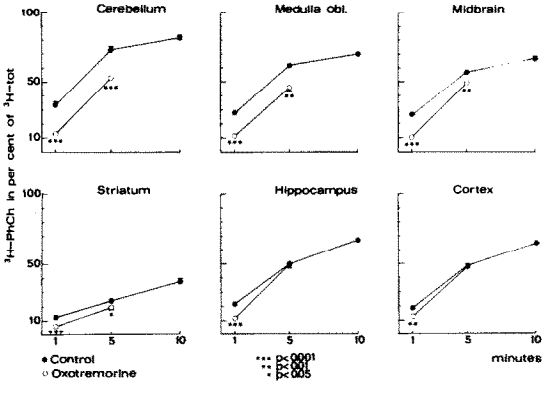

Fig. 3. Effect of oxotremorine (1 mg/kg) on the biosynthesis of [^{3}H]phosphorylcholine (^{3}H-PhCh) in different brain regions. The mice were sacrificed 1, 5, or 10 min after an intravenous injection of [^{3}H]choline (15 nmoles). Each point represents the mean value of 3–5 experiments. Vertical bars indicate S.E.

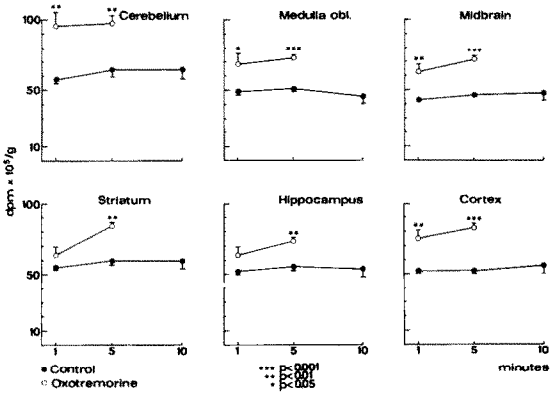

Fig. 2. Effect of oxotremorine (1 mg/kg) on the uptake and distribution of radioactivity (^{3}H-tot) in different brain regions. The mice were sacrificed 1, 5, or 10 min after an intravenous injection of [^{3}H]choline (15 nmoles). Each point represents the mean value of 3–6 experiments. Vertical bars indicate S.E.

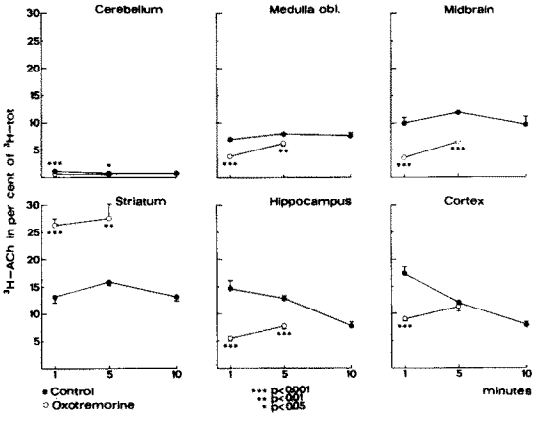

Fig. 4. Effect of oxotremorine (1 mg/kg) on the biosynthesis of [^{3}H]acetylcholine (^{3}H-ACh) in different brain regions. The mice were sacrificed 1, 5, or 10 min after an intravenous injection of [^{3}H]choline (15 nmoles). Each point represents the mean value of 3–6 experiments. Vertical bars indicate S.E.

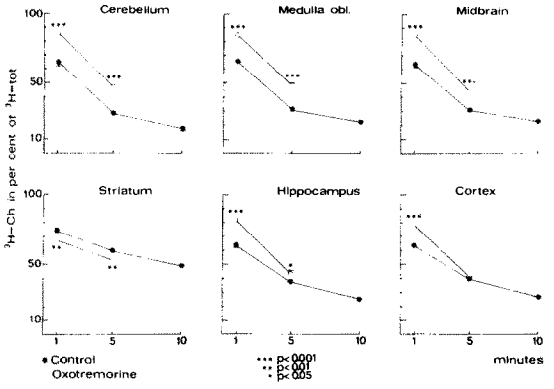

Fig. 5. Effect of oxotremorine (1 mg/kg) on [^{3}H]choline (^{3}H-Ch) in different brain regions. The mice were sacrificed 1, 5, or 10 min after an intravenous injection of [^{3}H]choline (15 nmoles). Each point represents the mean value of 3–6 experiments. Vertical bars indicate S.E.

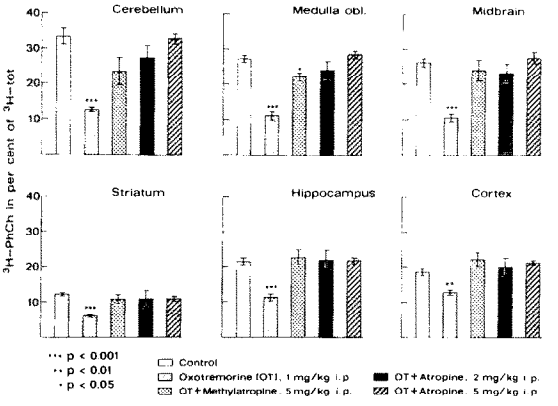

Fig. 7. Effect of oxotremorine (1 mg/kg) on the biosynthesis of [^{3}H]phosphorylcholine (^{3}H-PhCh) in different brain regions after pretreatment with methylatropine (5 mg/kg) and with atropine (2 or 5 mg/kg). The mice were sacrificed 1 min after an intravenous injection of [^{3}H]choline (15 nmoles). Each point represents the mean value of 3–6 experiments. Vertical bars indicate S.E.

that the rate of biosynthesis of [^{3}H]phosphorylcholine was reduced in the cerebellum, medulla oblongata, midbrain and striatum both at 1 and 5 min, compared with the control values. In the hippocampus and cortex the decrease at 1 min was followed by an increase to the control level at 5 min. Since oxotremorine enhanced the uptake of radioactivity in the brain, [^{3}H]phosphorylcholine, [^{3}H]acetylcholine and [^{3}H]choline are expressed as per cent of total radioactivity (^{3}H-tot) instead of as dis/min per g. The effect on [^{3}H]acetylcholine biosynthesis is shown in Fig. 4. In the cerebellum, medulla oblongata and midbrain the rate of this biosynthesis was decreased at both 1 and 5 min (22–64%). In the hippocampus and cortex it was decreased ($P < 0.001$) at 1 min but showed a slight tendency to increase between 1 and 5 min. At 5 min it was still significantly decreased ($P < 0.001$) in the hippocampus but was normal in the cortex. In the striatum the synthesis was *increased* by 74–101% ($P < 0.01$).

The content of untransformed [^{3}H]choline (Fig. 5) was significantly higher ($P < 0.001$) in the cerebellum, medulla oblongata and midbrain both at 1 and 5 min than in the controls. In the hippocampus and cortex it was significantly higher at 1 min ($P < 0.001$) but decreased markedly between 1 and 5 min; in the cortex this resulted in an insignificant difference ($P > 0.05$) at 5 min. In the striatum [^{3}H]choline was lower ($P < 0.01$) at both 1 and 5 min.

The ratio of [^{3}H]acetylcholine to [^{3}H]choline was reduced both 1 and 5 min after injection of oxotremorine in all brain regions except in the striatum, where it was increased ($P < 0.01$).

Figures 6–9 show the effects of oxotremorine after pretreatment with methylatropine (5 mg/kg) and with atropine (2 or 5 mg/kg). The animals were sacrificed 1 min after injection of [^{3}H]choline. The increased uptake of radioactivity obtained with oxotremorine alone was counteracted by both methylatropine and atropine. After treatment with 5 mg/kg of atropine

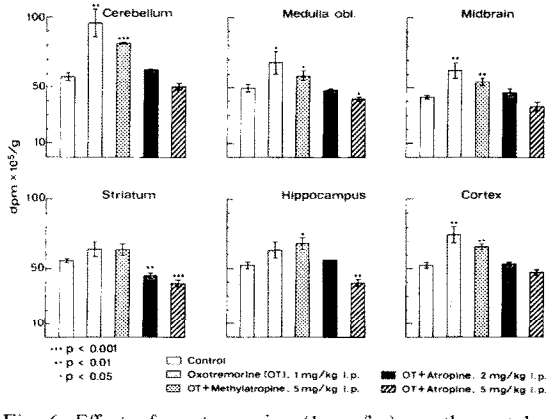

Fig. 6. Effect of oxotremorine (1 mg/kg) on the uptake and distribution of radioactivity (^{3}H-tot) in different brain regions after pretreatment with methylatropine (5 mg/kg) and with atropine (2 or 5 mg/kg). The mice were sacrificed 1 min after an intravenous injection of [^{3}H]choline (15 nmoles). Each point represents the mean value of 3–6 experiments. Vertical bars indicate S.E.

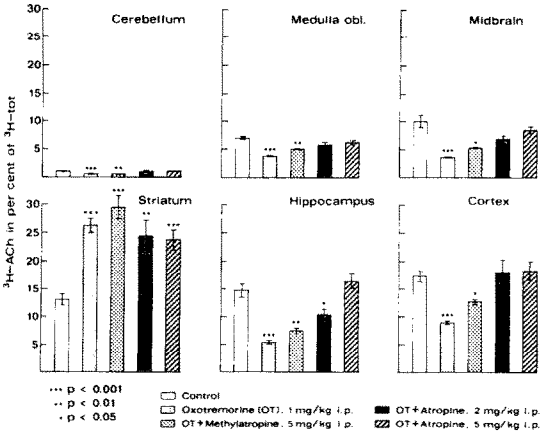

Fig. 8. Effect of oxotremorine (1 mg/kg) on the biosynthesis of [^{3}H]acetylcholine (^{3}H-ACh) in different brain regions after pretreatment with methylatropine (5 mg/kg) and with atropine (2 or 5 mg/kg). The mice were sacrificed 1 min after an intravenous injection of [^{3}H]choline (15 nmoles). Each point represents the mean value of 3–6 experiments. Vertical bars indicate S.E.

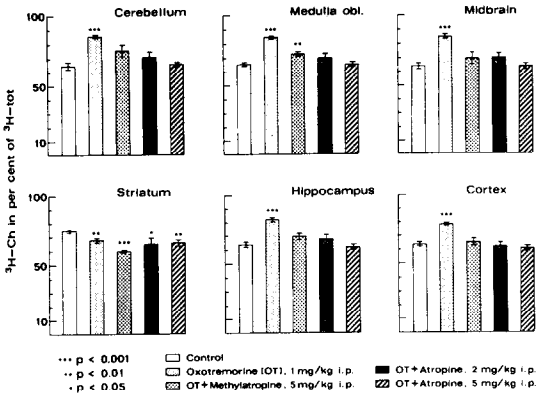

Fig. 9. Effect of oxotremorine on [^{3}H]choline (^{3}H-Ch) in different brain regions after pretreatment with methylatropine (5 mg/kg) and with atropine (2 or 5 mg/kg). The mice were sacrificed 1 min after an intravenous injection of [^{3}H]choline (15 nmoles). Each point represents the mean value of 3–6 experiments. Vertical bars indicate S.E.

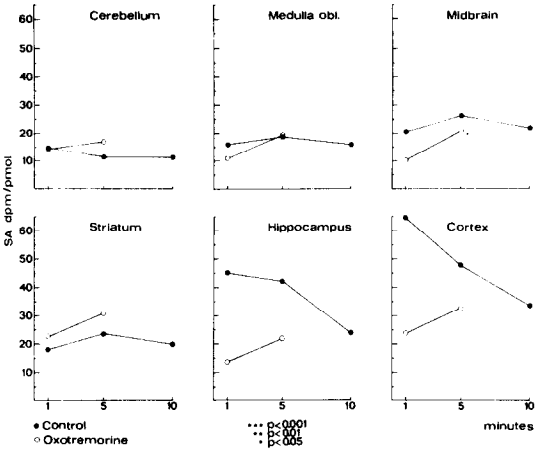

Fig. 10. Effect of oxotremorine (1 mg/kg) on the specific radioactivity (SA) of acetylcholine in different brain regions. The mice were sacrificed 1, 5, or 10 min after an intravenous injection of [^{3}H]choline (15 nmoles).

the total radioactivity was even lower than the control value in the striatum and hippocampus ($P < 0.01$) (Fig. 6). The reduced biosynthesis of [^{3}H]phosphorylcholine caused by oxotremorine was also counteracted by both methylatropine and atropine (Fig. 7). The reduced biosynthesis of [^{3}H]acetylcholine produced by oxotremorine was very little affected by pretreatment with methylatropine (Fig. 8); atropine (5 mg/kg), however, antagonized this reducing effect. In the striatum, where oxotremorine caused an increase in [^{3}H]acetylcholine biosynthesis, even this higher dose of atropine was ineffective. Pretreatment with methylatropine and with atropine antagonized the effects of oxotremorine on [^{3}H]choline in a similar way (Fig. 9).

Effect of oxotremorine on the specific radioactivity of acetylcholine. In Fig. 10 it is seen that oxotremorine decreased the specific radioactivity of acetylcholine particularly in the hippocampus and cortex. In the striatum the specific radioactivity was unchanged or possibly slightly increased. Since the specific radioactivities were calculated from the mean values of [^{3}H]acetylcholine and endogenous acetylcholine, no statistical analysis was possible.

Effect of oxotremorine on uptake and distribution of [^{3}H]dextran. Since oxotremorine appeared to facilitate the uptake of radioactivity in all brain regions, experiments were performed with radioactive dextran ([^{3}H]dextran) in order to test whether this enhancement could have been due to hemodynamic effects. It was found that the concentration of [^{3}H]dextran in the blood was 42% higher in the oxotremorine treated animals ($111{\cdot}6 \pm 4{\cdot}28 \times 10^{-5}$ dis/min per ml; $P < 0{\cdot}01$) than in the controls ($78{\cdot}4 \pm 1{\cdot}78 \times 10^{-5}$ dis/min per ml). The amount of [^{3}H]dextran found in the different brain regions, however, was directly related to the blood concentration (Table 3).

DISCUSSION

Our findings that administration of oxotremorine in the mouse increases the content of endogenous acetylcholine in the striatum, hippocampus and cortex but not in the cerebellum, medulla oblongata and midbrain are in agreement with observations in the rat by Campbell and Jenden [5]. In cat brain on the other hand, Bartolini *et al.* [6] noted a significant increase of endogenous acetylcholine in the diencephalon and upper part of the midbrain but no change in the cortex. *In vitro* oxotremorine (10^{-6}–10^{-5} M) produces a depression of the acetylcholine release from rat cerebral cortex slices following electrical

Table 3. Uptake and distribution of [^{3}H]dextran in different brain regions 10 min after intravenous injection

Brain region	dis/min per g brain tissue in per cent of dis/min per ml blood: Control	Oxotremorine 1 mg/kg, i.p.	Per cent* change
Cerebellum	3·0 ± 0·36 (3)	2·7 ± 0·33 (3)	−10·0
Medulla oblongata	3·0 ± 0·18 (3)	3·0 ± 0·42 (3)	±0
Midbrain	2·2 ± 0·22 (3)	2·1 ± 0·32 (3)	−4·5
Striatum	1·5 (1·7; 1·2)	1·2 ± 0·18 (3)	−2·0
Hippocampus	2·0 ± 0·10 (3)	2·1 ± 0·27 (3)	+5·0
Cortex	2·3 ± 0·14 (3)	2·2 ± 0·21 (3)	−4·3
Whole brain	2·4 ± 0·15 (3)	2·2 ± 0·31 (3)	−8·3

Oxotremorine, 1 mg/kg, was injected intraperitoneally 6 min prior to the dextran injection.
* $P > 0{\cdot}5$; (n) = number of experiments $M\bar{v} \pm$ S.E.

stimulation [12]. In the isolated perfused rat brain only a small and insignificant elevation of the level of endogenous acetylcholine occurred when the brain was perfused with 10^{-7} M oxotremorine [13]. It is stated in the latter report, however, that the lack of effect might have been due to too low concentration of oxotremorine since a higher concentration, 5×10^{-7} M, resulted in an increase from 31·6 to 39·8 nmoles/g acetylcholine in one out of seven brains.

The present results indicate that both atropine and methylatropine (5 mg/kg, i.p.) partially (50%) counteract the effect of oxotremorine (1 mg/kg) on endogenous acetylcholine in the cerebral cortex but have very little effect on acetylcholine in the striatum. That both drugs had similar effect on endogenous brain acetylcholine is surprising since they are supposed to penetrate the brain rather differently. The characteristic tremor produced by oxotremorine, on the other hand, was completely prevented by pretreatment with 5 mg/kg of atropine (i.p.) but not by 5 mg/kg of methylatropine and only partially by 2 mg/kg of atropine. Cox and Potkonjak [2] found no effect of 5 or 10 mg/kg of atropine on whole brain endogenous acetylcholine, while Sethy and van Woert [14] noted significant effects after administration of 10, 20, and 40 mg/kg. Holmstedt and Lundgren [15] reported complete inhibition after i.p. injection of 100 mg/kg of atropine.

The biosynthesis of [^{3}H]acetylcholine is decreased by oxotremorine in whole brain [3]. The marked increase of [^{3}H]acetylcholine biosynthesis in the striatum described in the present paper is masked in studies on whole brain due to the fact that the weight of the striatum is only 3% of the brain as a whole. Schubert *et al.* [3] have suggested that the effects of oxotremorine on endogenous and radioactive acetylcholine might be explained by the fact that oxotremorine is a muscarinic agonist, and that activation of muscarinic receptors would lead, by a negative feedback system, to depression of acetylcholine. This would initially increase the endogenous amount of the transmitter, whereupon synthesis would decrease. This could explain the findings in cerebral cortex and hippocampus. It is not possible at the present time to explain why oxotremorine increases both endogenous acetylcholine and the rate of biosynthesis of [^{3}H]acetylcholine in the striatum. This structure appears to be unique also in the respect that its cholinergic fibres almost completely consist of interneurons (for review see Aquilonius [16]).

In the cerebellum, medulla oblongata and midbrain the biosynthesis of [^{3}H]acetylcholine is decreased by oxotremorine without any concomitant effect on endogenous acetylcholine. It may be asked if this could be due to the low density of muscarinic receptors in these regions compared with cortex and striatum [17, 18].

The discrepancy between the effect of atropine and methylatropine on tremor and endogenous acetylcholine following treatment with oxotremorine is surprising. The influence of these drugs on the prevention of oxotremorine-induced tremor appears to be better related to the rate of biosynthesis of [^{3}H]acetylcholine. Since these antagonists were given prior to the agonist, their effects per se on endogenous acetylcholine and the rate of [^{3}H]acetylcholine biosynthesis must probably be taken into consideration.

The finding that oxotremorine increases the uptake of radioactivity in the brain is probably explained by hemodynamic effects. The blood concentration of [^{3}H]dextran was 42% higher in oxotremorine treated animals. This is in agreement with the findings of Karlén *et al.* [19] who previously found that oxotremorine decreases its own volume of distribution.

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