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Effect of oxotremorine and sodium pentobarbitone on the pharmacokinetics of intravenous tracer doses of radioactive choline

AGNETA NORDBERG

Department of Pharmacology, Faculty of Pharmacy, University of Uppsala, Box 573, S-751 23 Uppsala, Sweden

The present study explains for the previous findings that oxotremorine increases and sodium pentobarbitone decreases the initial brain uptake of intravenously injected radioactive choline (^{3}H–Ch). The effects are explained by haemodynamic changes since a corresponding increase and decrease in the plasma concentrations of ^{3}H–Ch were found. This is important to know when ^{3}H–Ch is used for estimation of acetylcholine turnover in the brain. The drugs did not affect the distribution of radioactivity (^{3}H) between plasma and erythrocytes.

The rate of formation of radioactive acetylcholine (^{3}H–ACh) in the brain from an intravenous tracer dose of radioactive choline (^{3}H–Ch) has been used as a method for the study of acetylcholine turnover in the brain (Schuberth, Sparf & Sundwall, 1969). Previous studies have shown that drugs may change the brain uptake of intravenously administered ^{3}H–Ch. Oxotremorine (1 mg kg^{-1}, i.p.) increases both the initial brain uptake of intravenously injected ^{3}H–Ch (+50%) and the brain concentration of unmetabolized ^{3}H–Ch (+80%) (Nordberg & Sundwall, 1976). Sodium pentobarbitone (60 mg kg^{-1}, i.p.) on the other hand, decreases the ^{3}H–Ch uptake (−20%) and the concentration of untransformed ^{3}H–Ch (−40%) (Nordberg & Sundwall, 1975). Since the content of newly synthesized ^{3}H–ACh (in d min^{-1} g^{-1}) in the brain varies with the brain uptake of ^{3}H–Ch, the cause of these changes has been investigated.

In the present study ^{3}H–Ch was given intravenously to animals pretreated with sodium pentobarbitone (60 mg kg^{-1}, i.p.) or oxotremorine (1 mg kg^{-1}, i.p.) and blood samples were taken at intervals of 15 s to 5 min after ^{3}H–Ch injection. Unmetabolized ^{3}H–Ch was isolated from plasma and measured, as well as the total radioactivity (^{3}H) of plasma and erythrocytes.

MATERIALS AND METHODS

Female mice (NMRI strain), 18–22 g, were housed for 3–6 days under constant diurnal illumination. They had free access to food and water. Tritium labelled choline [^{3}H]methylcholine, spec. act. 0·6 Ci mmol^{-1}) (15 n mol) was injected intravenously (in 2–3 s). From each animal one blood sample was taken from the infraorbital plexus at a time point of 15 s to 5 min after the ^{3}H–Ch injection. The actual time of collection was restricted to the period 5 s before to 5 s after the preselected time point. The blood samples were immediately centrifuged in polyethylene microtubes for 5 min (Beckman microfuge) to prevent *in vitro* equilibration of ^{3}H–Ch between plasma and erythrocytes. The plasma samples were extracted with an equal volume of ice-cold 10% trichloroacetic acid (TCA) and centrifuged. 20 μl of the extract was submitted to high voltage electrophoresis at pH 4·8 in pyridine–acetic acid–acetone–water buffer (8:8:30:154, by v/v). By this method it is possible to separate phosphorylcholine/betaine/lecithin from acetylcholine/betaine aldehyde and choline (Sparf 1973). Unlabelled choline was used as a carrier and visualized by iodine vapour. The radioactivity spots were localized by means of a radiochromatograph (Packard), eluted with water and counted by liquid scintillation (Nuclear Chicago Isocap 300; counting efficiency >60%). When all plasma had been carefully aspirated from the erythrocytes, the latter were weighed and oxidized in a sample oxidizer (Packard).

Oxotremorine oxalate (1 mg kg^{-1}, corresponding to 0·6 mg kg^{-1} free base) and sodium pentobarbitone (60 mg kg^{-1}, i.p.) were injected 15 min before the ^{3}H–Ch injection. In some experiments the animals were pretreated with atropine methylnitrate (5 mg kg^{-1}, corresponding to 4·2 mg kg^{-1} free base) and atropine sulphate (5 mg kg^{-1}, corresponding to 4·2 mg kg^{-1} free base) 15 min before the oxotremorine was given. The control animals received 0·9% saline. The rectal temperature was measured continuously during the experiments and hypo-

thermia was prevented by adjustment of an infra-red lamp.

RESULTS

Fig. 1 shows the plasma concentration of radioactive choline (^{3}H–Ch) following pretreatment with oxotremorine (1 mg kg^{-1}, i.p.), sodium pentobarbitone (60 mg kg^{-1}, i.p.) and saline. As seen, the plasma concentration of unmetabolized ^{3}H–Ch was 71% higher in the oxotremorine-treated animals 15 s after the injection than in the controls. The difference in concentration was still marked at 5 min (+75%). Sodium pentobarbitone, on the other hand, decreased the ^{3}H–Ch concentration in plasma. The decrease was most marked (−42%) at the earliest time point.

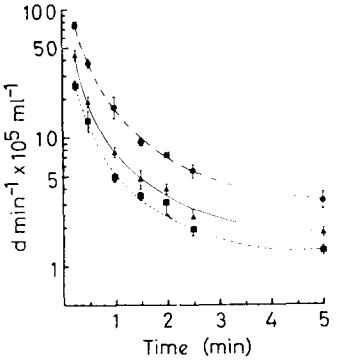

FIG. 1. The plasma concentration (d min^{-1} × 10^5 ml^{-1}) of unmetabolized radioactive choline (^{3}H–Ch) following pretreatment with ●-oxotremorine (1 mg kg^{-1}, i.p.), ■-sodium pentobarbitone (60 mg kg^{-1}, i.p.) and ▲-saline. Each point represents the mean value of 3–7 experiments. Vertical bars indicate s.e.

At 15 and 30 s the major part of the total radioactivity (^{3}H) in plasma was in the form of unmetabolized ^{3}H–Ch (85–90%) in all three groups. At 5 min about 50% was in the form of ^{3}H–Ch. The rest was localized to two spots corresponding to betaine/phosphorylcholine/lecithin and betaine aldehyde/acetylcholine. The uptake of ^{3}H in the erythrocytes was rather low. The concentration of ^{3}H in the erythrocytes was about 10 to 15% of that in plasma at all time points and was not significantly affected by the drug treatments.

Table 1 shows the plasma concentration of ^{3}H–Ch when the oxotremorine was preceded by methylatropine (5 mg kg^{-1}, i.p.) and by atropine (5 mg kg^{-1}, i.p.). The oxotremorine effect on the plasma ^{3}H–Ch concentration was not only prevented by both methylatropine and atropine but was also decreased by about 40% in comparison with the controls.

Table 1. *Effect of oxotremorine (1 mg kg^{-1}) on the plasma concentration of unmetabolized radioactive choline (^{3}H–Ch) after pretreatment with methylatropine (5 mg kg^{-1}) and with atropine (5 mg kg^{-1}).* The blood samples were taken 30 s after an intravenous injection of ^{3}H–Ch (15 nmol).

	Unmetabolized ^{3}H–Ch in plasma d min^{-1} × 10^5 ml^{-1}	% control	% ^{3}H	$\frac{A^* \times 100}{B^*}$
Control	19·1 ±1·55 (7)		85·3 ±0·84 (6)	10·6 ±2·31 (5)
Oxotremorine (1 mg kg^{-1}, i.p.)	38·2 ±2·78 (3)	200	88·5 ±0·80 (3)	9·1 ±0·41 (3)
Methylatropine (5 mg kg^{-1}, i.p.) + oxotremorine	10·4 ±1·46 (3)	54	57·5 ±2·96 (3)	14·3 ±2·83 (3)
Atropine (5 mg kg^{-1}, i.p.) + oxotremorine	12·4 ±1·89 (3)	65	63·5 ±0·25 (3)	11·8 ±1·57 (3)

* A = ^{3}H in erythrocytes. B = ^{3}H in plasma.
Mean value (Mv) ± s.e.
(n) = number of experiments.

DISCUSSION

The present study explains the previous findings that oxotremorine increases and sodium pentobarbitone decreases the initial brain uptake of intravenously injected ^{3}H–Ch and the brain concentration of unmetabolized ^{3}H–Ch (Nordberg & Sundwall, 1975, 1976). A corresponding increase and decrease in the plasma levels of unmetabolized ^{3}H–Ch were found. Thus it seems likely that the effects produced by the drugs on the brain uptake of ^{3}H–Ch are caused by haemodynamic effects. This was also indicated by the findings that oxotremorine increases both blood and brain concentration of intravenously administered [^{3}H] dextran similarly (Nordberg & Sundwall, 1976). In addition Karlén, Träskman & Sjöqvist (1971) showed by measuring the plasma concentration of [^{3}H]oxotremorine in animals treated with oxotremorine and with amitriptyline methyl iodide + oxotremorine, that oxotremorine might decrease its own volume of distribution.

Atropine and methylatropine are known to prevent the haemodynamic effects of oxotremorine. In addition the antiacetylcholine drugs *per se* have haemodynamic effects (Innes & Nickerson, 1970). All these effects together may explain the effects on plasma ^{3}H–Ch concentration observed in this paper.

Data from the literature indicate that barbiturates can produce vasodilatation, increase in blood flow and peripheral pooling of blood (for reviews see Faulconer & Patrick, 1957; Korner, Uther & White, 1968). In addition several investigators have found that administration of barbiturates to dogs in-

creases the plasma volume by about 9–12% and reduces the blood cell volume by about 6–12% (Bollman, Svirbely & Mann, 1938; Bonnycastle, 1942; Courtice & Gunton, 1949). Roentgenologic observations have shown that in dogs barbiturates cause an enlargement of the spleen and a reduction of the number of red cells in the peripheral blood (Hausner, Essex & Mann, 1938). In splenectomized dogs no change in cell volume was found after treatment with barbiturates compared with untreated animals (Bollman & others, 1938). In contrast, the spleen seems to have only a minor importance for the changes in plasma volume produced by barbiturates, since in splenectomized anaesthesized dogs the plasma volume was increased to the same extent as in dogs with an intact spleen (Bollman & others, 1938). These data may explain the decreased ^{3}H–Ch plasma concentration in anaesthesized mice, although the quantitative effects are more pronounced in the present study. This discrepancy might depend on species differences and/or the fact that the ^{3}H–Ch concentration in plasma was measured only short time points after the intravenous injection.

In studies *in vitro* by Martin (1968), it was observed that the human erythrocytes concentrated radioactivity from ^{14}C–Ch by a saturable mechanism (steady state ratio 2·08 at 2·5 μM and 0·94 at 50 μM). In the present *in vivo* experiment the ^{3}H in erythrocytes was only 10 to 15% of the ^{3}H in plasma at time points between 15 s to 5 min and the distribution between plasma and erythrocytes was not influenced by the drug treatments. The plasma concentration of choline in these experiments was in the range 6–12 μM. The difference between the experiments of Martin and this study is probably due to the longer incubation time used in the former study (16–20 h).

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