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Appearance in cat cerebrospinal fluid of radiolabelled metabolites following intraventricular injection of [^{3}H]choline

The metabolism of choline in brain tissue has been observed following intracerebral and intravenous administration (Ansell & Spanner, 1968; Diamond, 1971; Barker, Dowdall & Whittaker, 1972; Dowdall, Barker & Whittaker, 1972). We have injected [^{3}H]choline into the lateral cerebral ventricles of the cat and studied the appearance of radiolabelled metabolites in the effluent obtained during ventriculo-aqueductal perfusion.

Cats were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}) and mounted in a stereotaxic instrument. After cannulation of the right lateral ventricle and aqueduct, perfusion with artificial cerebrospinal fluid (csf) (in mM: Ca, 1·3; Na, 151·1, Mg, 0·9; K, 2·6; HCO_3, 21·0; HPO_4, 2·5; glucose, 3·4), thoroughly gassed with a mixture of CO_2 and oxygen, was begun at the rate of 100 μl min^{-1} with a Technicon auto-proportioning pump.

After 30 min, the perfusion was stopped, the outflow occluded and [^{3}H]choline chloride (30 μl; 1 μCi μl^{-1}; 17 Ci $mmol^{-1}$, Amersham Searle) injected. After 10 min, the perfusion was begun again with csf containing 1×10^{-4}M physostigmine sulphate. The effluent was collected at 5 min intervals, placed on ice in tubes containing 50 μl of 1×10^{-3}M neostigmine sulphate in acetate buffer (0·01 M), and either assayed immediately or frozen at −20°.

Aliquots were placed in Triton-toluene phosphor (TTP) and counted with a Packard Tri-carb liquid scintillation counter having a tritium counting efficiency of 20%. To separate lipids from water-soluble metabolites, 200 μl samples were mixed with 10 vol of a chloroform–methanol solution (2:1), capped, thoroughly agitated and allowed to stand for 12 h at 4°. The phases were then separated, the chloroform–methanol portion evaporated to dryness, TTP added and the samples counted. Water soluble metabolites were separated by high voltage electrophoresis (HVE). Authentic standards of choline, acetylcholine, betaine, and phosphorylcholine were run simultaneously and visualized with platinum-iodine reagent.

Fig. 1a illustrates the mean two phased decay curve obtained in 4 cats following the initiation of ventricular perfusion after injection of [^{3}H]choline chloride. Given that the effective volume of the cat ventricular system is about 10 ml, the results suggest that approximately 68% of the injected radioactivity remained in the ventricular space after 10 min. This rapid clearance of choline from the ventricular space was also observed by Aquilonius & Winbladh (1972). While the principal constituents in the early periods of the perfusion were water soluble (see Fig. 1b), there was a progressive increase in the relative proportion of lipid metabolites. At 240 min, the amount of recovered label incorporated into water-soluble metabolites and lipids was virtually identical.

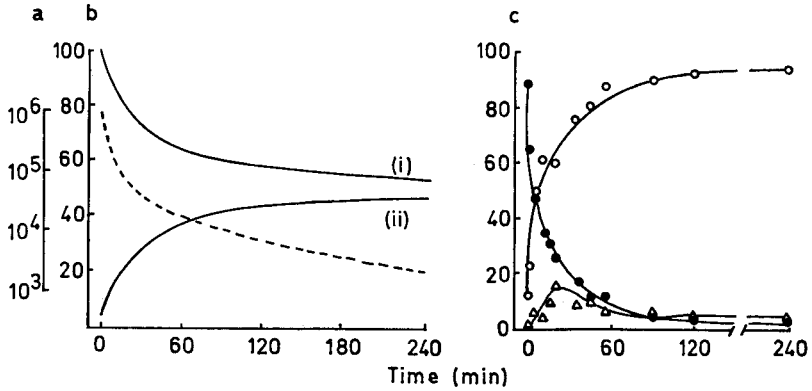

FIG. 1a. (---) Mean total radioactivity (counts min^{-1} ml^{-1}), in 4 cats, appearing in the ventricular effluent as a function of time after the start of perfusion. b. (—) Mean percent, in 4 cats, of total [^{3}H] label in the perfusion effluent incorporated into water soluble (i) and lipid soluble (ii) fraction as a function of time. c. Mean percent, in 4 cats, of total water-soluble radioactivity appearing in the perfusion effluent as choline (●), acetylcholine (△), or phosphorylcholine (○), as a function of time.

Although the [^{3}H]choline portion initially constituted over 95% of the total water-soluble fraction of the radioactivity in the effluent as found by HVE, this percentage fell markedly. The relative proportion of [^{3}H]phosphorylcholine showed a corresponding rise until by 1 h it represented over 80% of the water soluble fraction. The amount of [^{3}H]acetylcholine recovered was small (5–8%), except for a peak (10–15%) observed between 15 and 30 min after the initiation of perfusion. Betaine gave no significant peaks.

The rapid conversion of [^{3}H]choline into lipids in this system corresponds to the findings of Ansell & Spanner (1968) & Diamond (1971) for brain tissue although we observed the 50% conversion time to be 4 h in contrast to ½–2 h as reported by the previous workers. This difference may reflect the time required for the movement of [^{3}H]choline into tissue and the subsequent movement of the newly labelled lipids into the ventricular space. These data also concur with the observation of other investigators that phosphorylcholine appears to be the principal water-soluble metabolite of exogenously administered radio-labelled choline (Ansell & Spanner, 1968; Diamond, 1971; Dowdall & others, 1972). The percentages of labelled acetylcholine released into the cerebroventricles in the present experiments agree with the findings of Chakrin & Shideman (1968) in the cat cortex.

The absence of metabolites following incubation of [^{3}H]choline with csf indicates the absence of significant enzyme activity for acetylation or phosphorylation.

The amount of radiolabel recovered in the first perfusion sample 10 min after intraventricular injection of [^{3}H]choline in cats having high spinal sections (C-1) and artificially ventilated did not differ significantly from effluent activity observed in anaesthetized cats ($9{\cdot}6 \pm 0{\cdot}8 \times 10^5$ counts min^{-1} ml^{-1} *vs* $8{\cdot}9 \pm 1{\cdot}1 \times 10^5$ counts min^{-1} ml^{-1} student's *t*-test statistic; $P > 0{\cdot}10$). These results differ from those of other investigators who found marked reductions in uptake into brains of anaesthetized, as compared to unanaesthetized, animals following intravenous injection of radiolabelled choline (Schuberth, Sparf & Sundwall, 1969; Diamond, 1971). An explanation may be that all of our animals were maintained at a somewhat lower temperature (36·0–36·5°). Alternatively, it may be that the uptake system postulated for the transfer of choline from blood to brain may not function with respect to the movement of choline from csf into brain in the cat.

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Resistance of intestinal α-adrenoceptors to cold storage

It has been suggested that storage of rabbit intestine at 6–8° for 24 to 72 h leads to selective impairment of α-adrenoceptor function (Lum, Kermani & Heilman, 1966; Lum, Heilman & Gaunt, 1967; Salimi, Kermani & others, 1970).

In the present experiments the α-agonist (−)-phenylephrine and the β-agonist (−)-isoprenaline have been compared as inhibitors of the spontaneous isometric contractions in fresh and cold-stored segments of rabbit intestine, in the presence and absence of the selective antagonists, phentolamine and propranolol. The bathing solution used in the experiments was a modified Krebs solution with the following composition (mM):—NaCl 119·6, KCl 4·95, $CaCl_2$ 2·45, $MgSO_4$ 1·2, NaH_2PO_4 1, sucrose 10, glucose 10·9, $NaHCO_3$ 25.

A complete dose-response curve for either agonist was determined on a fresh jejunal segment. One of the two antagonists was then added to the bathing solution and after an equilibration period of 30 min the dose-response curve determination for the agonist was repeated. A similar procedure was subsequently followed with an adjacent segment which was stored at 6–8° for 24 h. The concentrations of antagonists which we used were those used in the experiments of Lum & others (1966). The potencies of the agonists have been expressed as pD_2 values (Ariens & van Rossum, 1957) where the maximal response was arbitrarily defined as complete extinction of at least one spontaneous contraction. The mean pD_2 values so obtained are shown in Table 1.

In fresh tissues, isoprenaline yielded log dose-response curves flatter than those produced by phenylephrine, the onset of its inhibitory action was slower and its effects lasted longer; however, its potency was similar to that of phenylephrine. These findings are in general agreement with those of van Rossum & Mujic (1965) and of Bowman & Hall (1970), and with the hypothesis of a dual adrenoceptor mechanism subserving inhibition in rabbit jejunum (Ahlquist, 1948; Ahlquist & Levy, 1959; Levy 1959; Furchgott, 1960).

Storage of tissues at 6–8° for 24–48 h did not affect the rate of spontaneous movement (15 min^{-1} for tissues equilibrated at 37°). In cold-stored tissues, the inhibitory potencies of both isoprenaline and phenylephrine were increased; there was less variability in tissue response; log dose-response curves were flatter; the onset and duration of the effects were indistinguishable from those observed in fresh tissues.

Phentolamine proved to be somewhat more potent an antagonist of phenylephrine in cold-stored tissues than in freshly excised tissues; thus the pA_2 (molar) estimates calculated from the results shown in Table 1 were 8·2 and 9·2 for fresh and cold-