**SHORT COMMUNICATION**

**Transport of lignocaine by rabbit choroid plexus in vitro**

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**Summary**

1. Lignocaine readily passes from blood into cerebrospinal fluid. The isolated rabbit choroid plexus, a locus of the blood–cerebrospinal fluid barrier, accumulated [14C]lignocaine by two processes: an active, saturable transport process and a non-saturable process.

2. The accumulation of [14C]lignocaine by choroid plexus was not due to non-specific binding or metabolism of lignocaine within or on the choroid plexus.

3. These results suggest that the choroid plexus may transport lignocaine between blood and cerebrospinal fluid in vivo by a specific carrier-mediated process.

**Key words:** active transport, choroid plexus, lignocaine.

**Introduction**

Lignocaine, whose intravenous use is limited by toxicity to the central nervous system, readily enters cerebrospinal fluid from the blood (Usubiaga, Moya, Wikinski, Wikinski & Usubiaga, 1967). Five minutes after the intravenous injection of lignocaine in man, the concentration in intracerebroventricular cerebrospinal fluid is 77% of the concentration in arterial blood and approximately 62% of the concentration in plasma (Usubiaga *et al.*, 1967; Covino & Vassallo, 1976). When the plasma protein binding of lignocaine of 64% is taken into account (Covino & Vassallo, 1976), the ratio of cerebrospinal fluid lignocaine to unbound plasma lignocaine is approximately 2:1.

The explanation offered for the very rapid appearance of lignocaine in cerebrospinal fluid is that the non-ionic form of lignocaine is lipid soluble and readily passes into cerebrospinal fluid (Usubiaga *et al.*, 1967; Rapoport, 1976). The partition coefficient (n-heptane/pH 7.4 buffer) for lignocaine is 2.9 (Covino & Vassallo, 1976). However, in plasma (pH 7.4), only 30% of lignocaine is non-ionic since the pKₐ of lignocaine is 7.7 (Covino & Vassallo, 1976). Hence, only that fraction of non-ionic lignocaine that is not protein-bound could freely penetrate from plasma into cerebrospinal fluid (pH 7.3) (Rapoport, 1976).

An alternative explanation for the appearance of lignocaine in cerebrospinal fluid would be that lignocaine is transported, in part, from plasma into cerebrospinal fluid by a carrier-mediated transport system. A possible location for the carrier-mediated transport of lignocaine from blood to cerebrospinal fluid is the choroid plexus, the anatomical locus of the blood–cerebrospinal fluid barrier (Lorenzo & Spector, 1976; Rapoport, 1976). The choroid plexus is known to transport some substances by specific carrier-mediated saturable processes from blood into cerebrospinal fluid (e.g. ascorbic acid, morphine) and from cerebrospinal fluid into blood (e.g. iodide, penicillin and choline) (Wang & Takemori, 1972; Lorenzo & Spector, 1976; Rapoport, 1976). Generally, substances that are transported between blood and cerebrospinal fluid by these carrier-mediated transport processes are accumulated by the isolated choroid plexus in *vitro* (Lorenzo & Spector, 1976).

The purpose of the present study was to investigate whether the isolated rabbit choroid
plexus contained an energy-dependent, saturable accumulation system for lignocaine.

Methods
The ability of isolated rabbit choroid plexuses to accumulate $^{14}$Clignocaine (30 mCi/mmol; New England Nuclear, Boston, Massachusetts, U.S.A.) was measured by methods previously described in detail (Spector, 1975). Individual choroid plexuses (weighing about 6 mg) were obtained from the brains of New Zealand white rabbits (1.5–2.0 kg) that were killed with intravenous pentobarbital (Spector, 1975). Each choroid plexus was placed in 3 ml of artificial cerebrospinal fluid containing glucose (5 mmol/l) and $[^{14}]$Clignocaine and, in some cases, other substances. The incubations were carried out in a metabolic shaker at 37°C or 1°C under O$_2$/CO$_2$ (95:5) for various times. At the end of the incubation, each choroid plexus was wiped on a glass slide, weighed, and homogenized in 0.5 ml of water. The radioactivity in tissue homogenates and media was determined (Spector, 1975), and the ratios of tissue to medium radioactivity were calculated by dividing the d.p.m./g of choroid plexus by the d.p.m./ml of medium.

The ability of choroid plexuses that had accumulated $[^{14}]$Clignocaine to release the $[^{14}]$Clignocaine was tested. In these experiments, after incubation for 2 min in $[^{14}]$Clignocaine (1-2 $\mu$mol/l) at 37°C, the choroid plexuses were rinsed in artificial cerebrospinal fluid (2 s) and transferred for a 10 min incubation into 2 ml of artificial cerebrospinal fluid containing lignocaine (0.1 mmol/l) at 37°C. The percentage of $^{14}$C in the release medium divided by the total $^{14}$C in the tissue and release medium was determined.

The purity of the $[^{14}]$Clignocaine and the possibility of metabolism of $[^{14}]$Clignocaine within the choroid plexus after 5 min incubations in artificial cerebrospinal fluid containing $[^{14}]$Clignocaine (1-2 $\mu$mol/l) were determined chromatographically (New England Nuclear).

To rule out further the possibility of non-specific binding of $[^{14}]$Clignocaine to choroid plexus, rabbit choroid plexuses were incubated for 2 min in $[^{14}]$Clignocaine (1-2 $\mu$mol/l) and subsequently homogenized in 1-0 ml of sodium chloride solution (154 mmol/l) (Lorenzo & Spector, 1973). Duplicate aliquots (0-1 ml) of supernatant, obtained after centrifugation at 1800g for 10 min, and homogenate were assayed for $^{14}$C.

Results
The time courses of accumulation (expressed as tissue to medium radioactivity ratios) of $[^{14}]$Clignocaine at 1°C and 37°C are shown in Fig. 1. At 37°C, the mean tissue to medium ratio at 2 min (7.81) was 85% of the 45 min value. The tissue to medium ratios at 1°C remained below 4.1 at all times tested (Fig. 1). Various substances, including lignocaine, tolazoline, glycinexylidide and dinitrophenol, significantly inhibited $[^{14}]$Clignocaine accumulation by the isolated choroid plexus. The mean tissue to medium ratios at 2 min in medium containing $[^{14}]$Clignocaine (1-2 $\mu$mol/l) and various substances, as a percentage of control, were: lignocaine (0-1 mmol/l), 75%; lignocaine (2-0 mmol/l), 47%; glycinexylidide (0-1 mmol/l), 84%, (2-0 mmol/l), 56%; tolazoline (1-0 mmol/l), 62%; probenecid (0-1 mmol/l), 109%; dinitrophenol (1-0 mmol/l), 50%. All values less than 63% were significantly different from controls ($P < 0.05$).

There was no significant metabolism of $[^{14}]$Clignocaine within the choroid plexus or medium. There was also no non-specific binding of the $[^{14}]$Clignocaine within the choroid plexus when measured by the centrifugation technique. In the supernatants of the choroid plexuses which had accumulated $[^{14}]$Clignocaine and were homogenized in saline, the concentration of $^{14}$C in the supernatant divided by the concentration of $^{14}$C in the homogenate was 1.01 ± SEM 0.02 ($n = 6$). This lack of non-specific binding within the choroid plexus was confirmed by the finding that 99 ± SEM 1% ($n = 3$) of the $[^{14}]$Clignocaine within the choroid plexus was released into the release medium in the 10 min release incubations.

Discussion
The principal finding reported herein is that the isolated choroid plexus concentrates lignocaine by
two processes. Greater than 50% of the uptake (with a medium concentration of 1.2 μmol/l) was by a saturable process and presumably depended on energy-production. Approximately 50% of the accumulation was by a non-saturable process and was not inhibited even at 1°C. If the pH values of the intracellular and extracellular spaces were 7.0 and 7.3 respectively (Lorenzo & Spector, 1973; Rapoport, 1976; Johanson, 1978), a ratio of 1.3 would be expected since the pKₐ of lignocaine is 7.7 (Covino & Vassallo, 1976) and the intracellular space of the choroid plexus represents 60% of its volume (Lorenzo & Spector, 1973). However, in these studies, a ratio of approximately 10 was obtained at equilibrium, indicating that other factors beside pH partitioning (active transport, for example) must be operating.

The possibility of non-specific binding of the lignocaine within or on the choroid plexus as the principal reason for the rapid accumulation of [¹⁴C]lignocaine by choroid plexus is not supported by the centrifugation studies (which showed no non-specific binding) and the ready release of 99%. Also the rapid accumulation of [¹⁴C]lignocaine by choroid plexus was not due to metabolism since chromatography of alkaline ethanolic extracts of choroid plexus on silica-gel thin-layer plates (in two systems) revealed no lignocaine metabolites.

The system in choroid plexus that accumulates lignocaine is inhibited by other weak bases, e.g. glycinexylidide, a lignocaine metabolite, and tolazoline, but not by probenecid, which inhibits the weak organic acid transport system of choroid plexus in vivo and in vitro (Spector & Lorenzo, 1974). The relationship between the saturable [¹⁴C]lignocaine-accumulation system and the accumulation systems in choroid plexus for primary, tertiary and quaternary amines and polyamines remains to be established (Spector, 1975; Rapoport, 1976; Lorenzo & Spector, 1976).

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References