Inhibition of 5-hydroxyindoleacetic acid transport from the spinal fluid by probenecid

It is generally accepted that 5-hydroxyindoleacetic acid (5-HIAA) in brain ventricular fluid derives from the metabolism of 5-hydroxytryptamine (5-HT) in the brain (Guldberg & Yates, 1968). Probenecid blocks active transport of 5-HIAA from ventricular fluid to blood and this is followed by an increase of 5-HIAA in ventricular and consequently in cisternal fluid (Guldberg, Ashcroft & Crawford, 1966). This active transport mechanism could be located in the choroid plexus (Ashcroft, Dow & Moir, 1968) or in the ependyma of the ventricles. We have observed that 5-HIAA in the fluid of spinal subarachnoid space (spinal fluid) derives from metabolism of 5-HT in the spinal cord. Since neither ependymal tissue nor structures similar to choroid plexus are present in the spinal subarachnoid space, the question arises of how 5-HIAA is removed from the spinal fluid. In these preliminary experiments we report that transport of 5-HIAA from spinal fluid can be inhibited by probenecid.

Experiments were made on adult cats lightly anaesthetized with thiopentone sodium. After thoraco-lumbar laminectomy, an extradural ligature of the spinal cord was placed at the T_{11} segment and saline (control) or probenecid (200 mg/kg) were administered intraperitoneally. Two h later, a sample of the cisternal fluid, obtained by percutaneous puncture of the cisterna magna, as well as a sample of the spinal fluid below the ligature were taken for analysis of 5-HIAA (Ashcroft & Sharman, 1962). Although mixing of cisternal and spinal fluid is negligible (Davson, 1967), the ligature was made to prevent any potential access of 5-HIAA from cisternal into spinal fluid below the ligature, as well as withdrawal of cisternal fluid into the syringe during the sampling of the spinal fluid. The ligature itself did not influence normal concentrations of 5-HIAA in cisternal and spinal fluid.

Table 1. *Concentrations of* 5-HIAA *in cisternal and spinal fluid* (ng/ml) *2 h after intraperitoneal application of probenecid* (200 mg/kg) *or saline (control).*

	5-HIAA (ng/ml)			
	Saline	Probenecid	Increase %	*t*-Test
Cisternal fluid	120 ± 10* (4)	250 ± 7 (3)	207	$P < 0{\cdot}001$
Spinal fluid	103 ± 5 (4)	226 ± 11 (4)	221	$P < 0{\cdot}001$

*Mean ± standard error of the mean.
Figures in parentheses represent number of experiments.

Table 1 shows that 2 h after probenecid, 5-HIAA significantly increases in both the cisternal and spinal fluid. Increase of 5-HIAA in the cisternal fluid after probenecid (Table 1) supports previous finding that 5-HIAA is actively transported from ventricular fluid to blood (Guldberg & others, 1966). Concomitant augmentation of 5-HIAA in the spinal fluid (Table 1) indicates that 5-HIAA is also actively transported out of the spinal fluid. Our results, therefore, suggest that the active transport system for the removal of 5-HIAA out of cerebrospinal fluid is not exclusively located in the choroid plexus or ependyma, since such a mechanism may exist in the spinal subarachnoid space. It has been shown recently that radioactive iodide may also be actively transported from spinal fluid into blood (Lorenzo, Hammerstad & Cutler, 1970).

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REFERENCES

ASHCROFT, G. W., DOW, R. C. & MOIR, A. T. B. (1968). *J. Physiol., Lond.*, **199**, 397–425.

ASHCROFT, G. W. & SHARMAN, D. F. (1962). *Br. J. Pharmac. Chemother.*, **19**, 153–160.

DAVSON, H. (1967). *Physiology of the cerebrospinal fluid*, pp. 152–158. London: J. & A. Churchill Ltd.

GULDBERG, H. C., ASHCROFT, G. W. & CRAWFORD, T. B. B. (1966). *Life Sci.*, **5**, 1571–1575.

GULDBERG, H. C. & YATES, C. M. (1968). *Br. J. Pharmac.*, **33**, 457–471.

LORENZO, A. V., HAMMERSTAD, J. P. & CUTLER, R. W. P. (1970). *J. neurol. Sci.*, **10**, 247–258.

"Free" and "bound" acetylcholine concentrations in rat brain: variability in determination of "free" acetylcholine fraction

Crossland & Slater (1968) have reported a method for simple fractionation of the brain acetylcholine into "free" (extracted with eserinized saline) and "bound" (extracted with acid-ethanol) components and have described the effect of some drugs on these fractions. Although the identity and the physiological significance of these fractions was not clear, they were, nevertheless, differentially affected by various groups of drugs.

We now describe our experience with determination of the "free" acetylcholine fraction using a modified approach.

Male Sprague-Dawley rats, 180–220 g were killed by dipping into liquid nitrogen for 10 s ("near-freezing" method of Takahashi & Aprison, 1964). Freezing and subsequent thawing of brain tissue under this condition does not occur. After decapitation the brains were carefully removed from the skull and weighed rapidly. The "free" and "bound" acetylcholine from the whole brain (without cerebellum, pons and medulla) were then extracted according to the procedure of Crossland & Slater (1968), or in other experiments, the "total" fraction from whole brain was extracted with acid-ethanol using the method of Crossland (1961). Assays were performed using the frog (*Rana temporaria*) rectus abdominis muscle sensitized with eserine sulphate ($1{\cdot}6 \times 10^{-5}$ M). Samples of tissue extracts were tested in a double-bracketed assay against standard solution of acetylcholine iodide prepared in alkali-inactivated parts of the same extracts (Feldberg, 1945). The recovery of acetylcholine added to the tissue homogenate was 90%.

During the bioassays we consistently noticed that the response of the frog rectus to the "free" acetylcholine samples declined after the first exposures of the muscle to this extract. This was not so when the acid-ethanol extracted samples of "bound" or "total" acetylcholine were assayed. This observation suggested to us that the direct estimation of the "free" fraction from the supernatant, obtained after extraction of the brain tissue with eserinized saline may yield false low values. We therefore decided to estimate directly the amount of "total" and "bound" fractions, both extracted with acid-ethanol from pooled opposing halves of the brains of a pair of animals ($R_1 + L_2$ for "total", $R_2 + L_1$ for "bound"). The values for the "free" fractions were then calculated by subtracting the values of "bound" from the values of "total" acetylcholine. The results are in Table 1.