Interaction of salicylate and cortisol on lysosomes

The stabilizing action of cortisol on lysosomes is abolished in the presence of salicylate (Lewis, 1970). In this communication we have examined the effect of salicylate on the uptake of cortisol by lysosomes.

The method employed has been described by Symons, Lewis & Ancill (1970). The procedure consists of immersing dialysis bags, containing rat or rabbit liver lysosomes in suspension in 0·05M tris-acetate buffered 0·25M sucrose (pH 7·4), into [^{3}H]-labelled steroid solutions prepared in the same sucrose buffer. In one set of experiments sodium salicylate to a final concentration of 5×10^{-4}M was added to the medium surrounding the dialysis bags.

Acid phosphatase and β-glucuronidase activity was measured in the supernatant obtained from centrifuging the contents of the dialysis bag at 20 000 *g* for 20 min at 4° (Symons, Lewis & Ancill, 1969). The protein concentration of the lysosome suspensions was determined by the method of Lowry, Rosebrough & others (1951).

The results (Table 1), show clearly that the presence of salicylate in the medium inhibited the uptake of cortisol by the organelles prepared from rabbit liver. A similar result was obtained with the organelles obtained from rat liver (Table 2).

Table 1. *The effect of salicylate on the uptake of cortisol by organelles in the dialysis bag.* The source of the organelles was rabbit liver. The results are the mean of two determinations.

Cortisol concn M	Salicylate concn M	Uptake of cortisol per mg protein n mole
5×10^{-3}	nil	35·2
5×10^{-3}	5×10^{-4}	23·9
5×10^{-5}	nil	0·660
5×10^{-5}	5×10^{-4}	0·318

Table 2. *The effect of salicylate on the uptake of steroids by organelles in the dialysis bag and the release of acid phosphatase from lysosomes.* Each result is the mean value $\pm$ s.e. of three determinations. The source of the organelles was rat liver.

Cortisol concn M	Salicylate concn M	Uptake of cortisol per mg protein n mol	% Release of lysosomal enzymes compared with control values adjusted to 100%	
			Acid phosphatase	β-Glucuronidase
$2·5 \times 10^{-3}$	nil	68·0 ± 4·1	101·2 ± 1·3	102·8 ± 2·0
$2·5 \times 10^{-3}$	5×10^{-4}	38·3 ± 3·1	92·7 ± 2·3	96·9 ± 1·1
$2·5 \times 10^{-4}$	nil	5·3 ± 0·5	96·9 ± 1·8	91·4 ± 2·4
$2·5 \times 10^{-4}$	5×10^{-4}	1·8 ± 0·2	100·3 ± 1·4	101·8 ± 1·1
$1·25 \times 10^{-5}$	nil	0·38 ± 0·03	90·8 ± 3·1	91·4 ± 1·1
$1·25 \times 10^{-5}$	5×10^{-4}	0·12 ± 0·03	100·3 ± 1·0	102 ± 2·7
$1·25 \times 10^{-6}$	nil	0·042 ± 0·003	95·4 ± 2·1	92·6 ± 1·0
$1·25 \times 10^{-6}$	5×10^{-4}	0·010 ± 0·002	103·1 ± 1·9	101·3 ± 2·1
$0·63 \times 10^{-7}$	nil	0·0016 ± 0·0002	101·2 ± 2·0	100·5 ± 1·0
$0·63 \times 10^{-7}$	5×10^{-4}	0·0004 ± 0·0001	102·2 ± 2·3	99·6 ± 1·6

In addition, the presence of salicylate has clearly abolished the stabilizing action of the cortisol on the lysosomes.

It would appear that salicylate inhibits the action of cortisol on lysosomes by inhibiting the entry of the steroid into the membrane.

A grant from the Royal Society for the purchase of a liquid scintillation counting system is acknowledged.

Pharmacology Group,
School of Pharmacy,
University of Bath,
Bath, Somerset, U.K.

D. A. LEWIS*
G. REED

August 24, 1971

* Present address, Department of Pharmacy, The University of Aston in Birmingham, Gosta Green, Birmingham, U.K.

REFERENCES

LEWIS, D. A. (1970). *J. Pharm Pharmac.*, **22**, 909–912.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). *J. biol. chem.*, **193** 265–275.

SYMONS, A. M., LEWIS, D. A., & ANCILL, R. J. (1969). *Biochem. Pharmac.*, **18**, 2581–2582.

SYMONS, A. M., LEWIS, D. A. & ANCILL, R. J. (1970). *J. Pharm. Pharmac.*, **22**, 944–945.

Effect of eserine injected intraventricularly on behaviour and on activity of cholinesterase in some structures of the cerebral ventricles of the conscious cat

The role of cholinesterase of various brain structures in behavioural changes after intraventricular injection of drugs acting on cholinoceptors is ill-understood. We now report the inhibition of acetylcholinesterase activity in the structures underlying the cerebral ventricles and relate this with the appearance of gross behavioural changes after intraventricular injection of eserine.

Cats of either sex, 1·9–4·4 kg were anaesthetized with pentobarbitone sodium (30–40 mg/kg, i.p.) and a cannula (Feldberg & Sherwood, 1953) was screwed into the skull through which injections were made in conscious cats. Eserine was injected intraventricularly in a volume of 0·2 ml and saline, 0·2 ml, was injected into the cerebral ventricles of controls. Thirty min after injecting eserine or saline the animals were decapitated. Brains were removed and immediately chilled in ice. Acetylcholinesterase activity of slices (Milošević & Andjelković, 1966), 0·5 mm thick, of superficial layers of caudate nucleus, thalamus, anterior and posterior hypothalamus, was measured manometrically in the Warburg apparatus (Umbreit, Burris & Stuffer, 1957). Acetylcholine (0·01 mM, final concentration) was the substrate. Enzyme activity was expressed in μl carbon dioxide liberated per mg of fresh tissue.

By increasing the dose of eserine, the degree of the inhibition of acetylcholinesterase activity was simultaneously increased in the caudate nucleus (Table 1). Acetylcholinesterase activity was inhibited with the highest doses of eserine in the thalamus and hypothalamus.

With a small dose of eserine (0·02 mg), when the acetylcholinesterase activity was inhibited in the hypothalamus, itching and sometimes ataxia developed.