An effect of ibuprofen and prednisolone on lysosomes

The effect of ibuprofen and prednisolone on lysosomes has been studied by Lewis (1970) and Lewis, Symons & Ancill (1970). By measuring the release of β-glucuronidase and acid phosphatase (A.P.) it was shown that prednisolone stabilized rabbit liver lysosomes in the concentration range 10^{-4}–10^{-8}M. With the less specific method of protein release ibuprofen had no effect on rat liver lysosomes. Ibuprofen inhibited free A.P. activity. We have studied the effect of these two drugs on rat liver lysosomes by measuring the release of A.P. and β-acetyl glucosaminidase (A.G.A.).

The method of Lewis & others (1970) was used to determine the stability of the lysosomes at 37°. The A.P. activity was assayed by the method of Caygill & Pitkeathly (1966) and the A.G.A. activity by the method of Woollen, Heyworth & Walker (1961).

The effects of the drugs on free A.P. and A.G.A. activities were also determined. A suspension of lysosomes in 0·05M tris-acetate buffered 0·25M sucrose (pH 7·4) was freeze-thawed several times, the debris removed by centrifugation at 20 000 *g* for 20 min at 4° and the supernatent diluted to give enzyme activities comparable to those released by the lysosomes in the control tubes in the stability experiments. Aliquots of this dilute enzyme preparation were added to flasks containing the appropriate amount of drug evaporated to dryness from portions of 1,4-dioxan solutions and were incubated at 37° for 90 min with shaking (100 oscillations/min). Activities were assayed and expressed as a percentage of those in control samples containing no drug.

Ibuprofen (10^{-5}–10^{-3}M) had no effect on A.P. or A.G.A. activity. However, 10^{-3}M prednisolone (but not 10^{-5} and 10^{-4}M) decreased the free activity of both enzymes (≈88% of control value). Fig. 1A shows that 10^{-3}M ibuprofen caused a significant reduction in lysosomal membrane stability ($P < 0.005$ for A.P. and $P < 0.001$ for A.G.A.). At 10^{-4}M there was a significant stabilization with respect to A.P. activity ($P < 0.05$) but no significant differences for A.G.A. At 10^{-5}M a significant stabilization was detected ($P < 0.01$) when measuring A.G.A but not when measuring A.P.

Fig. 1B (uncorrected for the effect of 10^{-3}M prednisolone on free A.G.A. and A.P. activity) shows that 10^{-3}M prednisolone caused a significant reduction in lysosomal stability ($P < 0.001$ for both enzymes). At 10^{-4}M there was no significant difference between tests and controls and at 10^{-5}M there was a significant stabilization ($P < 0.05$) for both enzymes.

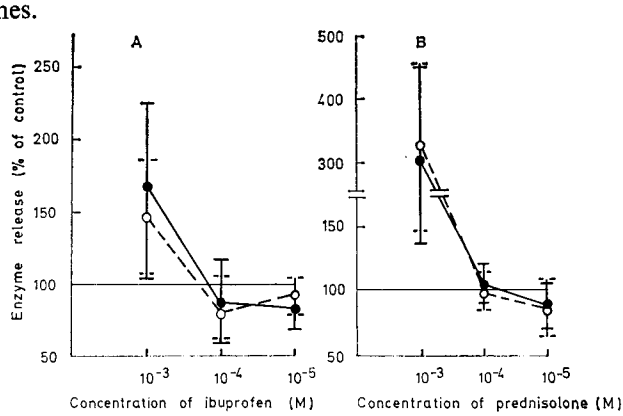

FIG. 1. The effect of (A) ibuprofen, (B) prednisolone on lysosomes. ○ -- ○ = acid phosphatase; ●—● = β-acetyl glucosaminidase. Each value is the mean of 12 values from a total of 4 experiments.

Thus in contrast to other studies we have found a stabilizing action of ibuprofen at 10^{-4} and 10^{-5}M on rat liver lysosomes, no effect of ibuprofen on free A.P. activity and an inhibition of free A.P. by 10^{-3}M prednisolone. Although significant, the stabilizing effect of these two drugs on rat liver lysosomes is slight and is unlikely to account for their full anti-inflammatory activity. Also it is curious that these *in vitro* effects of ibuprofen and prednisolone should be so similar in view of their differing potencies clinically.

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Increased accumulation of [^{3}H]catecholamines formed from [^{3}H]dopa after treatment with caffeine and aminophylline

There is growing evidence that the methylxanthines, caffeine and theophylline affect the metabolism of cerebral catecholamines. An increased turnover of noradrenaline brought about by these drugs has been reported (Berkowitz, Tarver & Spector, 1970; Waldeck, 1971; Corrodi, Fuxe & Jonsson, 1972). Further, the turnover of brain dopamine appeared first to increase and then to decrease after the administration of caffeine (Waldeck, 1971; Corrodi & others, 1972). The present study shows that the accumulation of [^{3}H]catecholamines formed from [^{3}H]dopa in the brain and heart of the mouse increases after treatment with caffeine and aminophylline.

Female mice, about 20 g, received an intraperitoneal injection of 100 mg/kg caffeine or aminophylline 30 min before the intravenous injection of 20 μg/kg L-dopa ring-2,5,6-^{3}H (The Radiochemical Centre, Amersham). Control animals received [^{3}H]dopa only. Sixty min after the labelled dopa had been given the animals were killed by decapitation, their brains and hearts removed and extracted in perchloric acid. [^{3}H]Noradrenaline (^{3}H-NA) [^{3}H]dopamine, [^{3}H]normetanephrine and [^{3}H]methoxytyramine were isolated with a combination of alumina and Dowex 50 columns. The analytical procedure and the testing of the radiochemical purity of the labelled dopa has been described by Persson & Waldeck (1968, 1970).

Caffeine increased the net accumulation of ^{3}H-NA and [^{3}H]dopamine in the brain by 80 ($P < 0.01$) and 130 ($P < 0.001$) % respectively (Fig. 1) while aminophylline tended to increase their yields though less markedly ($0.10 > P > 0.05$ and $P < 0.05$ respectively). [^{3}H]Normetanephrine appeared to follow the same pattern as ^{3}H-NA, however, statistical significance was not obtained. [^{3}H]Methoxytyramine in the brain increased threefold in animals treated with caffeine ($P < 0.001$) but was unchanged by aminophylline.

In the heart, the accumulation of ^{3}H-NA increased by some 30% after both caffeine and aminophylline ($P < 0.005$). [^{3}H]Dopamine was slightly increased ($P < 0.005$ after caffeine). There were no significant changes in [^{3}H]normetanephrine while the [^{3}H]methoxytyramine level doubled after caffeine ($P < 0.01$).

An increased accumulation of [^{3}H]catecholamines formed from [^{3}H]dopa can be brought about in various ways, e.g. by an increased transport of dopa into the adrenergic neuron, by an increased rate of decarboxylation or, by a decreased metabolism of the amines formed. None of these alternatives can be ruled out in