Biochemical and pharmacological properties of some amidopyrine metabolites

SIR,—A number of non-steroidal anti-inflammatory drugs uncouple oxidative phosphorylation, by selectively inhibiting the biogenesis of adenosine-5′-triphosphate (ATP) coupled to mitochondrial oxidative processes, without impairing cellular respiration (Adams & Cobb, 1958; Whitehouse, 1963, 1964a). Amidopyrine (4-dimethylaminoantipyrine, pyramidone) appears to be the exception to the hypothesis that this biochemical property coincides with anti-inflammatory activity, since neither amidopyrine itself nor two of its principal metabolities in man (4-aminoantipyrine and its *N*-acetyl derivative) uncouple oxidative phosphorylation at drug levels which have some resemblance to those used clinically (Whitehouse, 1963). We have now examined this point further by testing 4-aminoantipyrine (4-aminophenazone) and two other possible metabolities of amidopyrine (Jaffe, 1901; Proscher, 1902; Pechtold, 1964), namely rubazonic acid and *N*-methylrubazonic acid, for anti-inflammatory activity and ability to uncouple oxidative phosphorylation.

Drug action on phosphorylating rat liver mitochondria was studied by previously described methods (Whitehouse, 1964b) with sodium succinate as the substrate for mitochondrial respiration. 4-Aminoantipyrine and 4-nitrosoantipyrine had no effect on the phosphorylation quotient (P/O ratio) at 3 mM. Rubazonic acid, 10 μM, and *N*-methylrubazonic acid, 200 μM, inhibited phosphorylation (mitochondrial ATP biosynthesis) by approximately 50% without affecting oxygen uptake. Rubazonic acid is therefore rather more potent than either phenylbutazone or indomethacin (Whitehouse, 1964a) in uncoupling oxidative phosphorylation.

A modified ultra-violet erythema technique (Winder, Wax, Burr, Been & Rosiere, 1958) and the cotton pellet granuloma assay (Bush & Alexander, 1960) were used to assess the anti-inflammatory activity of these compounds in guinea-pigs and rats respectively. To study analgesic, antipyretic and anti-inflammatory (anti-oedema) activities in rats simultaneously, a procedure based on the method of Randall & Selitto (1957) was used. Anti-squirming ("analgesic") activity and ability to inhibit dye-release (anti-inflammatory) were also investigated in mice (Whittle, 1964). Antipyretic activity was investigated after the intravenous injection of 0·1 ml of a 1–4 dilution of T.A.B. vaccine in rabbits (Baker, Hayden, Marshall, Palmer & Whittet, 1963).

Amidopyrine and 4-aminoantipyrine were significantly active at 200 mg/kg by mouth in the Randall & Selitto test whereas rubazonic and *N*-methylrubazonic acids showed significant activity only in the lowering of the elevated paw temperature at 400 mg/kg orally. Amidopyrine, 4-aminoantipyrine and rubazonic acid did not significantly reduce the granuloma in the cotton pellet granuloma assay, when each was administered orally at 200 mg/kg/day for 7 days. (Other results are given in Table 1).

These results show that three oxidation products (metabolites) of amidopyrine are less active than amidopyrine itself as anti-erythema, anti-oedema and analgesic agents. These pharmacological assays also distinguish between amidopyrine and 4-aminoantipyrine on the one hand and the rubazonic acids on the other but there is no relation between potency in uncoupling oxidative phosphorylation and pharmacological activity.

These results suggest that *either* (1) the anti-inflammatory activity of amidopyrine is not due to its conversion *in vivo* to the rubazonic acids, even

TABLE 1. PHARMACOLOGICAL ACTIVITIES OF AMIDOPYRINE AND SOME OF ITS OXIDATION PRODUCTS (METABOLITES)

Compound	Ultra-violet erythema	Squirming test Relative activities		Antipyretic test
	Oral ED 50 mg/kg (confidence limits)	Reduction of squirms	Reduction of vascular permeability (dye-release)	Antipyretic index
Amidopyrine	16 (10 to 25)	1·00	1·00	27·0
4-Aminoantipyrine	24 (15 to 38)	0·62	0·77	13·0
Rubazonic acid	42 (24 to 79)	0·14	0·38	—
N-Methyl rubazonic acid ..	120 (55 to 260)	0·15	0·26	12

though the latter compounds (unlike amidopyrine) behave like many non-steroidal anti-inflammatory drugs in uncoupling oxidative phosphorylation, *or* (2) insufficient of the two rubazonic acids, when administered orally, reaches those sites where amidopyrine exerts its anti-oedema and anti-erythema properties. In view of the antihistaminic activity of amidopyrine (Domenjoz, 1960) and evidence that histamine is implicated in the inflammatory response (Bhatt & Sanyal, 1964), the first conclusion does not necessarily preclude the hypothesis that ability to uncouple oxidative phosphorylation determines anti-inflammatory activity in other drugs which, unlike amidopyrine, have little or no antihistaminic activity.

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