The antipyretic effect of α-methyldopa in experimental fever

α-Methyldopa (α-methyl-3,4-dihydroxy-1-phenylalanine) has a well-established position in antihypertensive therapy. The drug probably exerts its hypotensive effect via a central mechanism (Henning, 1969) probably after the conversion of α-methyldopa to α-methylnoradrenaline (Finch & Haeusler, 1972; Day, Roach & Whiting, 1972; Heise & Kroneberg, 1972). It was recently observed that after goats had been given α-methyldopa, 15 to 30 mg/kg (i.v.), the rise in body temperature to bacterial pyrogens was consistently reduced or abolished (van Miert, 1971).

We have now determined the dose-response relation after oral administration of α-methyldopa. Since endogenous pyrogen production by blood or tissue leucocytes has been clearly implicated as the cause of experimental fever due to bacterial pyrogens, viruses and antigen-antibody interactions (Atkins & Snell, 1965), the effect of α-methyldopa on fever induced with leukocytic pyrogen (LP) was also considered.

Tablets of α-methyldopa (250 mg) (Aldomet) were given orally to 25 newborn kids, 2·8 to 5·9 kg., because they resemble monogastric species in their digestive physiology, in contrast to adult goats. Kids also show very characteristic fever responses to both bacterial and leucocytic pyrogens (van Miert & Atmakusuma, 1971). Purified lipopolysaccharide (LPS) (0·2 μg/kg) from *Salmonella typhimurium** or leukocytic pyrogen (LP) (5 ml/kg ≡ 2×10^8 leucocytes/kg) was rapidly injected into the tarsal vein, respectively 4·5 and 6 h after the administration of α-methyldopa. Methods for maintaining glassware and solutions free of bacterial pyrogen contamination, for isolation peritoneal exudate cells, for preparing LP, and for fever testing in the recipient animals have been described by van Miert & Atmakusuma (1971) and van Miert, van Essen & Tromp (1972). The fever curves were plotted and a fever-index (F.I. 255) calculated; this being the area under the fever curve during 255 min of measurement.

After the injection of LPS in newborn kids, depression, piloerection and an increase of respiratory movements were observed after a short latency (< 30 min). Shivering, which occurred after 20 to 40 min, was biphasic like the temperature curve (Fig. 1B).

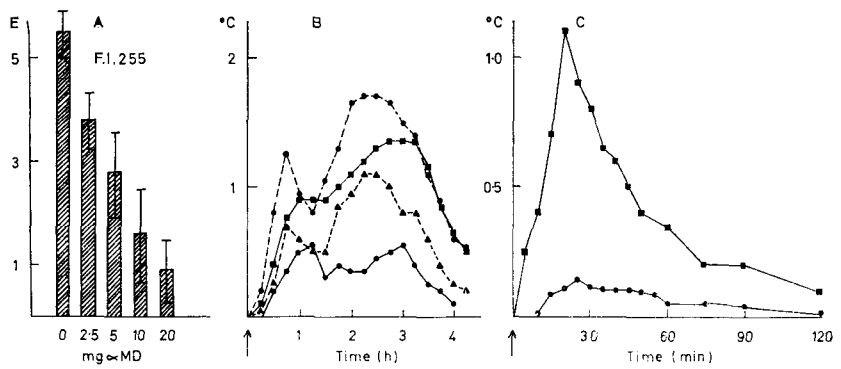

FIG. 1. A, B. The effect of α-methyldopa (α-MD) on LPS induced fever. Kids were pretreated orally with α-MD 4·5 h before i.v. injection of LPS 0·2 μg/kg. A: The mean fever index ± s.e. for groups of 5 kids given varying doses of α-MD as indicated. B: The effect of α-MD on the time course of LPS-induced fever. Each curve represents the mean rectal temperature of 5 kids receiving (mg/kg) α-MD 2·5 (■—■), 5 (▲--▲), 10 (●—●): the pyrogen only (●- - ●). C: Mean changes in rectal temperature of febrile kids before (■) and after (●) pretreatment with α-methyldopa (α-MD). The kids—a group of 4 animals—were pretreated orally with α-MD 20 mg/kg 6 h before i.v. injection of leukocytic pyrogen 5 ml/kg (equivalent to $2 \cdot 10^8$ leukocytes per kg).

* *S. typhimurium* batch 514553: Difco Laboratories, Detroit, Mich. U.S.A.

Pretreatment with α-methyldopa, orally caused a significant dose-related decrease in fever response to LPS (Fig. 1A) and shivering was inhibited or abolished. LP could be clearly differentiated from LPS by the nature of the febrile response which each elicited when injected intravenously. LP evoked a febrile response characterized by a shorter latency time, a more rapid rise to the peak height, a monophasic temperature curve and a quicker drop to the initial temperature level than that produced by bacterial pyrogens. Like LPS, the fever response to LP was reduced or abolished after a pretreatment with 20 mg α-methyldopa/kg (Fig. 1c).

The mechanism by which α-methyldopa lowers temperature during fever is not understood. On the other hand, α-methyl-*p*-tyrosine injected into the hypothalamus diminished the fever response due to LP administered into the preoptic area (Teddy, 1971). This compound inhibits the synthesis of noradrenaline and causes its depletion in the nerve endings in the hypothalamus. There is now little doubt that the endogenous monoamines noradrenaline and 5-hydroxytryptamine, which are found in relatively high concentrations in the hypothalamus, are involved at synapses concerned in the control of body temperature (Bligh, Cottle & Maskrey, 1971). Like noradrenaline, clonidine exerts an inhibitory influence at the hypothalamic synapses concerned in the control of body temperature (Maskrey, Vogt & Bligh, 1970). This drug lowered blood pressure by central α-adrenoceptor stimulation (Schmitt, Schmitt & Fénard, 1971). Dibenamine and tolazoline, which are α-adrenoceptor blocking agents, in contrast to β-adrenoceptor blocking drugs, blocked the temperature response to intracerebroventricularly administered noradrenaline (Dhawan & Dua, 1971). The temperature effect of imipramine and desipramine, which inhibit the uptake of noradrenaline by neurons, is indistinguishable from that of noradrenaline. Pretreatment with α-methyl-*p*-tyrosine antagonized the effect of both drugs (Cranston, Hellon & others, 1972). It is possible, therefore, that centrally acting drugs which cause disturbances to thermoregulation, exert their influence synaptically by mimicking the transmitter function or by affecting the synthesis, storage, release or destruction of a natural transmitter substance. High doses of α-methyldopa cause hypothermia in the rat (Garattini, Giachetti, & others, 1962). This drug exerts its blood pressure decreasing effect at least partly by stimulation of central α-adrenoceptors (Heise & Kroneberg, 1972). α-Methyldopa probably acts after conversion to α-methylnoradrenaline (Day & others, 1972; Finch & Haeusler, 1972). Although α-methylnoradrenaline simulates the temperature effect of noradrenaline to a minor degree, this compound effectively suppressed the temperature effect to subsequent intracerebroventricularly administered noradrenaline (Dhawan & Dua, 1971). It seems reasonable, therefore, to assume that the antipyretic effect of α-methyldopa is due to a central action of this drug. It is possible that false transmitters like α-methyldopamine and α-methylnoradrenaline are formed in the central nervous system and that these compounds interfere with thermo-regulation. This hypothesis is open to further research.

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In vitro release of endogenous histamine, together with noradrenaline and 5-hydroxytryptamine, from slices of mouse cerebral hemispheres

Evidence supporting the assertion that biogenic amines function as neurotransmitters in the central nervous system is much greater for noradrenaline, dopamine, 5-hydroxytryptamine (5-HT) and acetylcholine (Bloom & Giarman, 1968; Andén, Carlsson & Häggendal, 1969) than for histamine (Green, 1964; 1970). One of the characteristics to be expected of a neurohumour is that it should be stored in a readily-releasable form in neurons, and be released into the synaptic cleft in response to stimulation and depolarization of the nerve terminal membrane. Noradrenaline, dopamine and 5-HT have been shown to be stored in neurons of the central nervous system, and the release of these amines and acetylcholine has been demonstrated both *in vivo* and *in vitro*. Depolarization has been induced in brain slices by either electrical stimulation or increased concentrations of K^+ in the incubation medium (for review see Katz & Chase, 1970). Using the latter technique, we have recorded the release of endogenous histamine, noradrenaline and 5-HT from slices of mouse cerebral hemispheres.

The results now presented were obtained from a study primarily designed to observe the effects of different drugs on the metabolism of indoles in slices of mouse hemispheres and on the release of monoamines *in vitro*, as influenced by changes in the $[K^+]$ in the incubation medium. Histamine was determined in only a limited number of experiments.

Normal white female mice (NMRI), 18–24 g, were decapitated and the hemispheres (minus corpus striatum) were dissected, sliced and weighed. Six hemispheres were incubated for 40 min in 5 ml Krebs-Henseleit solution (Krebs & Henseleit, 1932) (equilibrated with 5% CO_2 in 95% O_2) which was modified by adding ethylene diamine tetra-acetate (EDTA), 15 μg/ml, and ascorbic acid, 20 μg/ml. Drugs were added for the purpose of the indole study and the $[K^+]$ was varied as indicated in Fig. 1. After incubation, the 'Krebs' was decanted and the slices blotted dry. In each experiment, two groups of six mice provided 24 hemispheres. Six hemispheres from each group were incubated in 'normal $[K^+]$-Krebs' and six in 'high $[K^+]$-Krebs', all other parameters being identical. Subsequently, the 'normal $[K^+]$-Krebs' from both groups were combined, as were the appropriate slices: the 'high $[K^+]$-Krebs' were similarly combined as were the slices (for further details see Carlsson, 1970). The proteins in the 'Krebs' solutions and in the slices were precipitated with perchloric