intervals) were 0·007 (0·006–0·009) μM (n = 3) and 0·720 (0·604–0·865) μM (n = 3), respectively. At the end of each experiment the α_2-adrenoceptor antagonist, yohimbine, and the dopamine receptor antagonist, haloperidol, were tested to reverse compound-induced inhibition. Yohimbine (0·28 μM) did not produce reversal but haloperidol (0·13 μM) completely reversed the inhibitory responses of both compounds. Thus these experiments indicate DA-2 receptor involvement and not of α_2-receptors.

Discussion

In this study, both enantiomers of 4-hydroxy-2-di-n-propylaminoindan were found to be active as inhibitors of stimulation-induced tachycardia through stimulation of presynaptic dopamine receptors, since the inhibitory effects of the compounds were completely reversed by the dopamine receptor antagonist haloperidol. These properties are similar to those described in previous reports in which apomorphine and other DA-2 receptor agonists were used (Ilhan & Long 1975; Ilhan et al 1976b). Also, reversal of compound-induced hypotensive and bradycardic responses by haloperidol indicates the involvement of dopaminergic mechanisms.

The present results show that the *R*-enantiomer of 4-hydroxy-2-di-n-propylaminoindan is 100 times more potent than its *S*-enantiomer in the stimulation of presynaptic dopamine receptors of cat hearts both in-vivo and in-vitro. Likewise, the *R*-enantiomer is potent in inducing contralateral rotations in rats with unilateral denervation of the nigro-striatal pathway. For rotational behaviour, the minimal effective dose for the *R*-enantiomer is 0·24 μmol kg^{-1} while the *S*-enantiomer exhibits comparable activity at 3·0 μmol kg^{-1}.

This work was supported in part by NIH research grant GM-22365. The spatial orientation of the isomers was determined by Dr Noel D. Jones, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA.

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J. Pharm. Pharmacol. 1985, 37: 365–366
Communicated October 4, 1984

Evidence against putrescine and polyamines as endogenous mediators of fever

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Endogenous concentrations of putrescine, spermidine, spermine and related biosynthetic enzymes were not affected by the administration of bacterial endotoxin and the subsequent development of fever in rabbits. In addition, the febrile response to endotoxin was unaffected either by the ornithine decarboxylase inhibitor, DL-α-difluoromethylornithine or by putrescine. These data indicate polyamines are not involved in the development of fever.

Putrescine, spermidine and spermine are naturally occurring amines which are intimately related with, if not modulators of nucleic acid and protein synthesis (Tabor & Tabor 1976). Protein synthesis is essential for the development and maintenance of fever in response to pyrogens such as bacterial endotoxins. Fever is mediated by a heat-labile endogenous protein produced by phagocytes such as polymorphonuclear leucocytes and monocytes (Gander 1982). Leucocytes contain negligible amounts of preformed pyrogenic protein whose synthesis has to be induced by agents such as bacterial endotoxin (Fessler et al 1961). In addition there is evidence, reviewed by Milton & Sawhney (1982), that the development of fever is dependent also upon the fresh synthesis of protein in the brain.

We set out to investigate the possible involvement of putrescine, spermidine and spermine in the pathogenesis of fever by measuring endogenous concentrations of these amines and related enzymes, and by determining the effects of DL-α-difluoromethylornithine (DFMO), a selective and irreversible inhibitor of ornithine decarboxylase (ODC) (Fozard & Koch-Weser 1982), and of putrescine on fever.

Methods

Established techniques described elsewhere (Dascombe 1984) were used to obtain and monitor pyrogenic responses to *Shigella dysenteriae* lipopolysaccharide in

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rabbits at an ambient temperature of 20–23 °C. DFMO (Merrell International) was dissolved in 0·9% NaCl (saline), putrescine dihydrochloride (Sigma Chemical Company) in water. Drug solutions were sterilized by filtration (pore size 0·22 μm) before injection either intraperitoneally (i.p.) or intravenously (i.v.). The saline, water, syringes and needles used were sterile, pyrogen-free commercial products.

Endogenous concentrations of putrescine, spermidine, spermine, N_1-acetyl-spermidine and N_8-acetyl-spermidine were assayed by post-column derivatization high performance liquid chromotography in tissues removed after death from rabbits receiving thiopentone sodium 50 mg kg^{-1} i.v. ODC and *S*-adenosyl methionine decarboxylase (SAM-DC) activities were assayed in the same tissue samples by the release of $^{14}CO_2$ from [1-^{14}C]-labelled substrates.

Fevers were assessed as changes in colonic temperature from pre-injection values (Δ °C) and as a temperature response index (TRI) integrating Δ °C against time (h). Results are expressed as the mean ± s.e.m. for n experiments. Differences between the means of experimental data have been evaluated by a 2-tailed Student's *t*-test for related or unrelated data as appropriate.

Results

Rabbits injected i.v. with *Shigella dysenteriae* lipopolysaccharide (LPS) 5 μg kg^{-1} had a significantly higher colonic temperature (40·1 ± 0·3 °C, n = 6, $P < 0·02$) at the time of death, 1 h after injection, than control rabbits receiving saline 0·2 ml kg^{-1} i.v. (39·3 ± 0·1 °C, n = 6). Endogenous concentrations of putrescine, spermidine and spermine in the preoptic/anterior hypothalamic region, the cerebral cortex, skeletal muscle, liver and plasma were unaffected by the administration of LPS and the development of fever. Although putrescine and spermidine were not detectable in plasma (<0·1 nmol ml^{-1}), there were detectable amounts of the metabolites N_1-acetyl-spermidine (8·8 ± 0·4 nmol ml^{-1}, n = 6) and N_8-acetyl-spermidine (11·9 ± 0·5 nmol ml^{-1}, n = 6) in control rabbits but concentrations of these compounds were not significantly different in LPS-treated rabbits. No ODC activity was found in samples from either afebrile or febrile rabbits, SAM-DC activity was evident but values were similar for both control and LPS-treated rabbits in all tissues studied.

DFMO (25, 100 or 400 mg kg^{-1}, n = 6 or 7) injected i.p. 1 h after LPS 5 μg kg^{-1} i.v. was without significant effect ($P > 0·1$) on the febrile response measured for 4 h after administration of DFMO. DFMO 200 mg kg^{-1} injected i.p. in rabbits 1 h before the pyrogen was also without effect ($P > 0·5$) on the febrile responses to doses of 1 μg kg^{-1} (TRI 3·48 ± 0·66 °C × h, n = 8) and 5 μg kg^{-1} (TRI 5·01 ± 0·54 °C × h, n = 8) over the 4 h following i.v. injections of LPS. DFMO 400 mg kg^{-1} produced vasodilatation in ear veins and a fall in colonic temperature (−0·4 ± 0·1 °C, n = 7) in afebrile rabbits.

Putrescine 100 mg kg^{-1} but not 10 mg kg^{-1} injected i.v. produced ear skin vasodilatation of rapid onset and a fall in temperature significantly different ($P < 0·02$) from the response of rabbits to vehicle. Hypothermia was maximal (−0·4 ± 0·1 °C, n = 8) about 50 min after injection of putrescine and lasted about 2 h. Putrescine 100 mg kg^{-1} injected i.v. 1 h after LPS 5 μg kg^{-1} i.v. had no effect on the febrile response to endotoxin ($P > 0·6$, n = 6). Higher doses of putrescine (500 and 200 mg kg^{-1}) were injected i.p. but were found to be irritant and toxic in initial studies with both control and LPS-treated rabbits (n = 2 or 3), consequently studies with these doses of putrescine were discontinued.

Discussion

Endogenous concentrations of putrescine, spermidine and spermine in the pyrogen-sensitive preoptic/anterior hypothalamic nuclei, the cerebral cortex, skeletal muscle, liver and plasma were unaffected by the administration of bacterial endotoxin and the resultant development of fever in rabbits. Levels of ODC activity, which decarboxylates L-ornithine to form putrescine, and SAM-DC, which decarboxylates *S*-adenosyl methionine to form the aminopropyl donor for spermidine and spermine synthesis from putrescine, were also unaffected by LPS-induced fever. These data indicate that putrescine, spermidine and spermine are not involved in the response of these tissues to bacterial endotoxin. The possibility exists that polyamine biosynthesis is altered by bacterial endotoxin in tissues other than those studied here but it is rendered less tenable by the observation that DFMO, a selective and irreversible inhibitor of ODC (Fozard & Koch-Weser 1982), injected either before or after administration of LPS was without effect on fever. In addition, exogenous putrescine injected into rabbits in sub-toxic doses had no significant effect on the febrile response to LPS, indicating not only that putrescine may not be involved in the development of fever but also that the diamine does not have antipyretic activity, although it is reported to be anti-inflammatory (Bird et al 1983). In conclusion the data presented here indicate that polyamines are not involved in the development of fever in rabbits.

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