Maj, J., Rogóż, Z., Skuza, G., Sowińska, H. (1984b) J. Neural Transm. 60: 273–282

Martin-Iverson, M. T., Leclere, J.-F., Fibiger, H. C. (1983) European J. Pharmacol. 94: 193–201

Pijnenburg, A. J. J., Honig, W. M. M., Van Rossum, J. M. (1975) Psychopharmacologia (Berlin) 41: 175–180

Pijnenburg, A. J. J., Honig, W. M. M., Van Der Heyden, J. A. M., Van Rossum, J. M. (1976) European J. Pharmacol. 35: 45–58

Spyraki, C., Fibiger, H. C. (1981) Ibid. 74: 195–206

Wędzony, K., Maj, J. (1983) 8th Congress of Polish Pharmacological Society, Warsaw, September 26–28, 1983, Abstract p. 181

J. Pharm. Pharmacol. 1985, 37: 364–365
Communicated September 6, 1984

© 1985 J. Pharm. Pharmacol.

Dopamine receptor agonistic activities of *R*- and *S*-enantiomers of 4-hydroxy-2-di-n-propylaminoindan in cat hearts

MUSTAFA ILHAN†, JAN R. FLYNN, JOHN P. LONG*, R. K. BHATNAGAR, JOSEPH G. CANNON, *Departments of Pharmacology* and Medicinal Chemistry, Colleges of Medicine and Pharmacy, The University of Iowa, Iowa City, Iowa 52242, USA*

In-vitro and in-vivo studies were used to evaluate the presynaptic dopamine receptor stimulating activities of *R*- and *S*-enantiomers of 4-hydroxy-2-di-n-propylaminoindan in cat hearts. Bioassay results show that the *R*-enantiomer is 100 times more potent than the *S*-enantiomer in both in-vitro and in-vivo preparations.

Hacksell et al (1981) reported that *RS*-4-hydroxy-2-di-n-propylaminoindan is slightly less active or equipotent to apomorphine in stimulating central dopamine receptors. Recently, Cannon et al (1984) synthesized *R*- and *S*-enantiomers of 4-hydroxy-2-di-n-propylaminoindan. Several reports using cat hearts have demonstrated that when presynaptic dopamine receptors (DA-2) are stimulated there is an inhibition of positive chronotropic responses produced by stimulating the right postganglionic cardioaccelerator nerves in both in-vivo and in-vitro experiments (Long et al 1975; Ilhan et al 1976a, b). We have evaluated the presynaptic dopamine receptor stimulating actions of *R*- and *S*-enantiomers of 4-hydroxy-2-di-n-propylaminoindan in cat hearts both in-vitro and in-vivo.

Method

In in-vivo experiments, cats were anaesthetized by i.p. injection of sodium pentobarbitone (30 mg kg^{-1}). The trachea was cannulated, the animal artificially respirated and the thorax opened by a midline incision. Right postganglionic cardioaccelerator nerves were placed on a bipolar electrode for stimulation. Arterial blood pressure was measured from the cannulated left femoral artery using a Statham pressure transducer (P23AA). Heart rate was monitored by a cardiotachometer which was triggered by the systolic pulse pressure. Solutions of experimental compounds were administered i.v. through a catheter placed in the right femoral vein and the arterial blood pressure and heart rate were recorded on a multichannel oscillograph (Beckman, Model R611). Cardioaccelerator nerves were stimulated for 30 s every 5 min with a Grass S48 stimulator at 20–30 V using a pulse width of 2 ms and a frequency of 2 Hz. After three consecutive reproducible responses to stimulation had been obtained, compounds were injected.

In in-vitro experiments, cats were anaesthetized as before. Following midline thoracotomy the heart was excised and the right atrium isolated and suspended between 2 platinum electrodes in a 100 ml organ bath containing Feigen solution (mM): NaCl 154·0; KCl 4·6; $CaCl_2$ 5·6; $NaHCO_3$ 23·8 and glucose 11·1. The atrium was transmurally stimulated at 2 Hz, 5 ms duration and 100 V for 10 s. Atrial rate was monitored with a Beckman Model 9857B cardiotachometer. Feigen solution was aerated with 95% O_2 and 5% CO_2 and maintained at 37 °C. A Statham force displacement transducer, Beckman recorder and a Grass S48 stimulator were used. IC50 values and 95% confidence intervals were determined by probit analysis as described by Finney (1952).

Results

In the in-vivo experiments, both compounds caused dose-dependent inhibition of the tachycardic response to cardioaccelerator nerve stimulation. The ID50 values (with 95% confidence intervals) of the *R*- and *S*-enantiomers are 0·011 (0·008–0·015) μmol kg^{-1} (n = 4) and 1·29 (0·62–15·8) μmol kg^{-1} (n = 4), respectively. Compound-induced inhibition of tachycardia during neuronal stimulation was completely reversed by haloperidol (0·13 μmol kg^{-1}) which also completely reversed the bradycardic and hypotensive response to the highest dose of compounds (data not shown). The *R*-enantiomer (0·03 μmol kg^{-1}) caused 37·0 ± 9·0% decrease of blood pressure and basal heart rate respectively (n = 4) while, the *S*-enantiomer (1 μmol kg^{-1}) lowered arterial blood pressure and basal heart rate by 30·1 ± 11·2% and 19·0 ± 8·1%, respectively (n = 4).

Both the *R*- and *S*-enantiomers produced inhibition of stimulation-induced heart rate increases in isolated cat atria and the IC50 values (with 95% confidence

† Present address: University of Hacettepe, Department of Pharmacology and Toxicology, Ankara, Turkey.
* Correspondence.

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.

REFERENCES

Cannon, J. G., Dushin, R. G., Long, J. P., Ilhan, M., Jones, N. D., Swartzendruber, J. K. (1984) J. Med. Chem. (In press)

Finney, D. J. (1952) Statistical Methods in Biological Assay, Charles Griffin and Co., London.

Hacksell, U., Arvidsson, L. E., Svensson, U., Nilsson, J. L. G., Wikström, H., Lindberg, P., Sanchez, D., Hjorth, S., Carlsson, A., Paalzov, L. (1981) J. Med. Chem. 24: 429–434

Ilhan, M., Long, J. P. (1975) Arch. Int. Pharmacodyn. Ther. 216: 4–10

Ilhan, M., Long, J. P., Cannon, J. G. (1976a) Ibid. 222: 70–80

Ilhan, M., Long, J. P., Cannon, J. G. (1976b) Ibid. 219–204

Long, J. P., Heintz, S., Cannon, J. G., Kim, J. (1975) J. Pharm. Exp. Ther. 192: 336–342

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

© 1985 J. Pharm. Pharmacol.

Evidence against putrescine and polyamines as endogenous mediators of fever

M. J. DASCOMBE*, K. D. HAEGELE†. *Department of Pharmacology, Materia Medica & Therapeutics, Medical School, University of Manchester, Oxford Road, Manchester M13 9PT, UK and †Merrell Dow Research Institute, Strasbourg, France*

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

* Correspondence.