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# The effect of (−)-4-(2-hydroxy-3(*N*-isopropylamino)-propoxyimino)-*cis*-carane on basal and forskolin-stimulated accumulation of cyclic AMP in the cerebral cortical slices of the rat

TADEUSZ LIBROWSKI, IRENA NALEPA*, RYSZARD CZARNECKI, JERZY VETULANI*, *Department of Pharmacodynamics, Copernicus Medical Academy, Podchorążych 1, 30-084 Kraków, and *Department of Biochemistry, Institute of Pharmacology P.A.N., Smętna 12, 31-343 Kraków, Poland*

**Abstract**—(−)-4-(2-Hydroxy-3(*N*-isopropylamino)-propoxyimino)-*cis*-carane, a local anaesthetic and platelet aggregation inhibitor which is much more potent than lignocaine, facilitated forskolin-induced cyclic (c) AMP accumulation in cerebral cortical slices of the rat. Lignocaine was ineffective in this respect. It is hypothesized that a cAMP-related mechanism may be involved in increased efficacy of the compound.

(−)-4-(2-Hydroxy-3(*N*-isopropylamino)-propoxyimino)-*cis*-carane (HNIPPC) was synthesized as a monoterpene propanol analogue and found to be a potent local anaesthetic, four times more potent than lignocaine in the infiltration anaesthesia test (Siemieniuk et al 1992). Further studies have demonstrated that HNIPPC potently inhibits platelet aggregation, being in this respect 20 times more potent than lignocaine (Czarnecki et al 1992).

Local anaesthetics are known to inhibit platelet aggregation, probably by interference with calcium transport (Feinstein et al 1976; Anderson et al 1981). On the other hand, platelet aggregation is known to be inhibited by cyclic (c) AMP (Kerry & Scrutton 1983; Winther & Trap-Jensen 1988). HNIPPC, being a monoterpene, shows some structural resemblance to the diterpene forskolin, a potent activator of adenylate cyclase (Seamon & Daly 1986) which inhibits platelet aggregation (Agarwal & Parks 1982); in the present study we compared the effect of lignocaine and HNIPPC on the cAMP second messenger system in the cerebral cortical tissue of rats.

## Materials and methods

The experiment was carried out on cerebral cortical slices obtained from male Wistar rats. The tissue was prepared as described by Nalepa & Vetulani (1991) and 300 μm slices were suspended in $O_2$:$CO_2$ (95:5)-gassed, glucose-containing modified Krebs–Henseleit medium (composition in mM: NaCl 118, KCl 5, $CaCl_2$ 1·3, $MgSO_4$ 1·2, $KH_2PO_4$ 1·2, $NaHCO_3$ 25, glucose 11·7, pH 7·4) at 37°C, which was used throughout all incubations.

cAMP accumulation was assayed by the method of Shimizu et al (1969) using a double column system according to Salomon et al (1974). The effect of HNIPPC (synthesized and donated by Professor Krzysztof Piątkowski of Wrocław Polytechnic) and lignocaine (Polfa, Kraków) on accumulation of cAMP was tested in the absence and in the presence of 1 μM forskolin (Sigma Chemical Company, St Louis, MO, USA).

## Results and discussion

As shown in Fig. 1, neither compound affected the basal level of cAMP. The stimulatory effect of forskolin on cAMP accumulation was unaffected by lignocaine, but was potentiated by higher concentrations of HNIPPC. The effectiveness of HNIPPC in this respect (EC50 = 380 μM) was of the same order of magnitude as its effectiveness in the anti-aggregatory test (EC50 = 260 μM, Czarnecki et al (1992)).

The present results suggest that at least a part of the anti-aggregatory action of HNIPPC results from its interference with the cAMP-generating system. The nature of potentiation of the effect of forskolin is presently unknown. A likely possibility is inhibition of cAMP phosphodiesterase, although a co-operation at the active site of adenylate cyclase cannot be excluded. Regardless of the detailed mechanism of action of HNIPPC, the present results suggest that the mechanism of its anti-aggregatory action differs from that of lignocaine, as it may involve mechanisms relating to the cAMP system.

We suggest that the higher potency of HNIPPC as a local anaesthetic, and particularly its much greater activity as an anti-aggregation agent, as compared with lignocaine, results in an additional, cAMP-related, mechanism of action of the former compound.

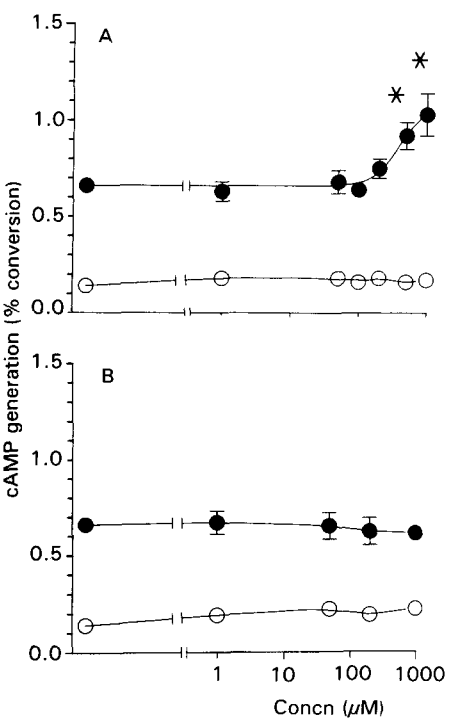


FIG. 1. The effect of HNIPPC (A) and lignocaine (B) on basal (○) and forskolin-induced (●) cAMP accumulation in rat cortical slices in-vitro. The data (%[$^3$H]adenine conversion) represent the stimulation (increase over the basal level of 0·14 ± 0·02%, n = 8). The points are means of 7–8 separate experiments carried out in duplicate. The bars represent s.e.m. Analysis of variance demonstrated significant differences between means ($F = 6·81$, df 6/57). *Significant potentiating effect ($P < 0·01$, Fisher's LSD test). EC50 for the stimulatory effect of HNIPPC, calculated from the sigmoid curve by nonlinear regression, was 381 μM.

Correspondence: J. Vetulani, Institute of Pharmacology P.A.N., Smętna 12, 31-343 Kraków, Poland.

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# Selective effects of pentamidine on cytosolic and granule-associated enzyme release from zymosan-activated human neutrophilic granulocytes

M. A. ARNOTT, N. D. BENNETT*, D. CAIRNS†, J. HAY‡, *Department of Medical Microbiology, The University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, *Department of Microbiology, The University of Leicester, Leicester LE1 9BA, †School of Health Sciences, Sunderland University, Sunderland SR2 7EE, and ‡Department of Microbiology, The Royal Infirmary, Glasgow G4 0SA, UK*

**Abstract**—Therapeutic concentrations (0·3–1·5 mg $L^{-1}$) of pentamidine isethionate, normally obtainable in-vivo after parenteral administration of the drug, did not affect the in-vitro activity of the enzymes lysozyme, β-glucuronidase or myeloperoxidase released from zymosan-activated human neutrophilic granulocytes. At concentrations of 0·7, 1·1 and 1·5 mg $L^{-1}$, activity of cytosolic enzymes lactate dehydrogenase and glucose-6-phosphate dehydrogenase were reduced relative to untreated cells ($P < 0.001$ and $P < 0.01$, respectively), but not in a dose-dependent fashion. Cell viability, as determined by dye-exclusion remained unaffected.

Pentamidine (1,5-bis (*p*-amidinophenoxy)-pentane) is an aromatic diamidine which is useful in the treatment and prophylaxis of pre-CNS trypanosomiasis and leishmaniasis (Sands et al 1985). The pharmaceutical importance of pentamidine has increased recently as a consequence of its activity against HIV-associated *Pneumocystis carinii* infection (Wispelway & Pearson 1991).

Pentamidine can compromise certain functions of neutrophilic granulocytes, at least in-vitro. At concentrations within the therapeutic range after administration of a standard parenteral dose of the drug in-vivo, there is a depression in the candidacidal capacity of stimulated neutrophilic granulocytes (Arnott & Hay 1989a), a phenomenon likely to result from a drug-induced depression of components of the respiratory burst of such cells (Arnott & Hay 1989b); the latter may, in turn, result from interference with the action of the membrane-associated NADPH-oxidase system of these phagocytic cells (Arnott & Hay 1990; Arnott et al 1991).

Correspondence: M. A. Arnott, Department of Medical Microbiology, University of Edinburgh, Edinburgh EH8 9AG, UK.

Degranulation is one of the major functions of neutrophilic granulocytes; the process results in the release of enzymes which are involved in oxygen-independent killing of microbes (Spitznagel & Schafer 1985).

The primary aim of the present study was to determine in-vitro whether therapeutic concentrations of pentamidine would interfere with degranulation of human neutrophilic granulocytes using the enzymes lysozyme, β-glucuronidase and myeloperoxidase as markers of the process. Activities of the cytosolic enzymes lactate dehydrogenase and glucose-6-phosphate dehydrogenase were also determined after exposure to pentamidine.

## Materials and methods

*Isolation of neutrophilic granulocytes.* Human neutrophilic granulocytes from five normal, healthy adult male donors were separated from heparinized venous blood by sedimentation for 45 min at 37°C with Plasmagel (Universal Biologicals Ltd) in a Plasmagel-to-blood ratio of 1:4 and processed according to the method of Babior & Cohen (1981). Only cell preparations with a cell viability and purity of ⩾98%, as determined by trypan blue exclusion and May–Grünwald–Giemsa staining, respectively, were used in the assays.

*Degranulation.* Neutrophilic granulocytes ($0·5–2·0 \times 10^7$) were preincubated with pentamidine for 30 min at 37°C. Drug concentrations of 0·3, 0·7, 1·1 and 1·5 mg $L^{-1}$, calculated in