# Structure-activity relation of pyrimidine nucleotides and nucleoside in canine isolated cerebral vessels*

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The vasoactivity of adenine nucleotides has been studied in a variety of vascular preparations (Burnstock, 1972). In contrast, relatively little is known about the vascular effects of pyrimidine nucleotides (PN). The present paper, of which a preliminary report has appeared elsewhere (Urquilla, Van Dyke & Trush, 1977) describes the contractile activity of pyrimidine nucleotides on isolated cerebral arteries of the dog, and extends some previous observations by Hashimoto Kumakura & Tanemura (1964) on other arteries.

Mongrel dogs of either sex (10 to 15 kg) were anaesthetized with sodium pentobarbitone (40 mg $kg^{-1}$, i.v.) and then exsanguinated. The brain was removed and the middle cerebral arteries dissected. Cylindrical segments of the vessels of approximately 5 mm length and 500 $\mu$m in outside diameter were set up for isometric recording in an organ bath according to Nielsen & Owman (1971). The bath contained 10 ml of Krebs-Henseleit solution at 37° bubbled with 5% carbon dioxide in oxygen. The recording system consisted of force-displacement transducers and a polygraph. A resting tension of 0·5 g was applied to the tissue and readjusted every 15 min during a 1 h equilibration. Dose-response curves for PN and uridine were determined in a cumulatively manner. Chemical substances used were: uridine 5′-triphosphate (UTP), uridine 5′-diphosphate (UDP), uridine 5′-monophosphate (UMP), uridine, cytidine 5′-triphosphate (CTP) and thymidine 5′-triphosphate (TTP). TTP was purchased from Nutritional Biochemical Corp., and the other substances were purchased from Sigma Chemical Co. Solutions of PN and uridine were prepared in saline immediately before use and kept surrounded by ice. Concentrations are expressed in molar terms.

The dose-response curves for the different PN and uridine are presented in Fig. 1. All agents were tested in the concentration range of $1·7 \times 10^{-6}$ to $1·7 \times 10^{-4}$M. The contractions induced by UTP, UDP, UMP and CTP were dose-dependent; UTP and UDP displayed the highest potency of all the agents tested. The potencies of UTP and UDP were identical and their maximal effects were, respectively, $1·57 \pm 0·12$ and $1·49 \pm 0·21$ g ($P>0·05$); these effects were 37·3 and 35·4%, respectively, of the response elicited with 150 mM of potassium chloride. These findings indicate that reduction of the phosphate chain by one phosphate group did not alter the potency of the parent compound (UTP), suggesting that hydrolysis of the high energy bond between the $\beta$- and $\gamma$-phosphate groups of UTP was not required for the action to take place. In some experiments, successive dose-response curves for UTP were determined and no evidence of desensitization was observed. Further shortening of the phosphate chain, such as occurs in the conversion of UDP to UMP, is accompanied by marked reduction in the contractile activity of the nucleotide. The absence of phosphate groups in the molecule, as in the nucleoside uridine, made the compound virtually inactive.

Hence, it appears that a phosphate chain of 2 or 3 phosphate groups is required for the nucleotide to be able to activate the contractile mechanism of the vessel. Structural alterations at the other end of the molecule, namely on the pyrimidine ring, were also accompanied by marked changes in contractile activity. Replacement of the keto group of the 4 position in the ring by an amino group, a change which converts UTP to CTP, was associated with decreased ability to induce contractions. Hindrance to the keto group of the 4 position in the ring by a methyl substituent in the 5 position, such as it occurs in TTP, abolished the

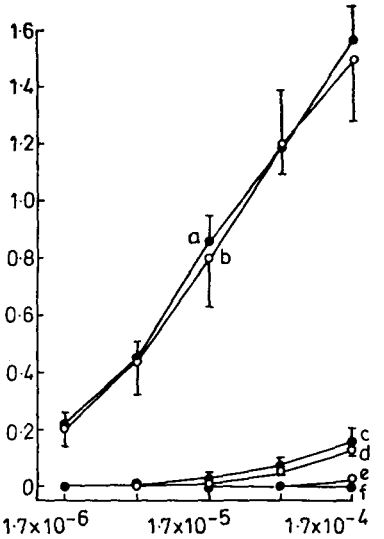


FIG. 1. Dose-response curves for pyrimidine nucleotides and nucleoside determined in the isolated middle cerebral artery of the dog. Vertical bars represent standard errors of the means. a—UTP (n=5). b—UDP (n=5). c—UMP (n=5). d—CTP (n=4). e—Uridine (n=4). f—TTP (n=3). Ordinate: Increase in tension (g). Abscissa: Dose (M).

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contract tile activity of pyrimidine nucleotides. These results suggest that the cerebral arteries of the dog can be stimulated to contract by UTP and UDP, presumably, through interaction with a tissue component or receptor system which appears to be complementary to the uracil part and to the phosphate chain (2 or 3 phosphates in length) of the pyrimidine nucleotide molecule.

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# Monoamine oxidase inhibitory properties of 5-hydroxymethyl-3-*m*-tolyloxazolidin-2-one (toloxatone)

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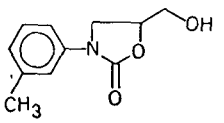
This paper reports on the monoamine oxidase (MAO, EC 1.4.3.4.) inhibiting properties *in vitro* and *in vivo*, of a new oxazolidin-2-one derivative with antidepressant activity in animals (Gouret, Mocquet & others, 1977) and potential clinical efficacy in man (Martin, 1973). A comparison has been made with clorgyline, a specific inhibitor of MAO A (Johnston, 1968) and (±)-deprenyl, a specific inhibitor of MAO B (Knoll & Magyar, 1972) (for a review see Houslay, Tipton & Youdim, 1976).




In vivo *studies*. Male Charles River rats, 125–150 g, were injected intravenously with drugs dissolved in saline and decapitated at preselected times after administration (Fig. 3). Their brains were rapidly removed and homogenized (Ultraturrax) in 10 volumes (w/v) of ice cold 0·2 M phosphate buffer pH 7·40. MAO activity was measured using $^{14}$C-5-HT ([2-$^{14}$C]-5-hydroxytryptamine creatinine sulphate, 0·43 $\mu$Ci, specific activity 54–58 mCi mmol$^{-1}$, the Radiochemical Centre, Amersham) or $^{14}$C-$\beta$-PEA ([1-$^{14}$C]-$\beta$-phenethylamine hydrochloride, 0·39 $\mu$Ci, specific activity 51 mCi mmol$^{-1}$, New England Nuclear) as substrates according to a procedure adapted from Wurtman & Axelrod (1963). Briefly: samples (0·5 ml) of brain homogenates were incubated at 37° in air with 0·45 ml of phosphate buffer and 0·050 ml of $^{14}$C-5-HT or $^{14}$C-$\beta$-PEA. Incubation times were respectively 10 and 5 min when MAO activity was linear with respect to both time and protein concentration. Then, 0·2 ml of 4 N HCl and 7 ml of toluene was added to each tube which was mechanically shaken (10 min) and centrifuged (3000 *g*, 5 min). Four ml of the upper organic phase was counted in 10 ml of a scintillation mixture of 2,5-diphenyl oxazole (PPO) in toluene (0·4%, w/v). The blank was boiled homogenate. Protein was measured according to Lowry, Rosebrough & others (1951), with bovine serum albumin as a standard.

For the measurement of concentrations of toloxatone and its metabolites in brain, groups of 3 rats were injected with the drug labelled with $^{14}$C in the carbonyl group (50 mg. kg$^{-1}$, 20 $\mu$Ci, specific activity 5 mCi mmol$^{-1}$, ICN Pharmaceuticals) and killed 5, 30, 60, 120 and 240 min later. Brains were rapidly removed, frozen at −20° and lyophilized. Samples (50 mg) were combusted in an oxidizer, the $^{14}CO_2$ absorbed in a scintillation mixture of methanol–toluene–phenethylamine–bidistillated water (22:40:33:50 v/v) containing PPO (0·4%), and the radioactivity measured by liquid scintillation counting.

In vitro *studies*. The specificity of the MAO inhibitory (MAOI) effect of toloxatone was compared *in vitro* with clorgyline HCl (M & B) and (±)-deprenyl HCl. Aliquots (0·5 ml) of whole rat brain homogenates in 10 volumes (w/v) of 0·2 M phosphate buffer pH 7·40 were preincubated at 22° for 15 min with various concentrations of MAO inhibitors in a total volume of 0·95 ml. MAO activity was assayed as already described using $^{14}$C-5-HT or $^{14}$C-$\beta$-PEA and the per cent inhibition plotted against inhibitor concentration using the log-probit representation. In these conditions, the concentration of a particular inhibitor producing 50% inhibition of MAO activity (IC 50) with either 5-HT or $\beta$-PEA as substrate, was determined graphically.

Kinetic parameters (Michaelis constant, Km; maximal velocity Vmax; inhibitory constant, Ki) of MAO inhibition by toloxatone were determined from double reciprocal plots using rat brain stems rapidly dissected

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