This is contrary to what we observed in an earlier study[14], when we administered para-methoxyphenylethylamine (PMPEA) to a group of 10 rats in chronic hypertensive stress. In that study[14], the blood pressure fell within 1 week from its maximum to a value of 133 mm Hg and then further to 122 mm Hg within the following week. Contrary to this type of immediate, and probably direct effect of PMPEA upon some central cardiovascular mechanism, diazepam appeared indirectly to prevent the stress from maintaining the blood pressure at the hypertensive level by a central filtering of the stressful stimuli. Since diazepam did hinder stressful stimuli from maintaining an elevated blood pressure in addition to preventing chronic stress-induced hypertension, it can be assumed that its effect upon blood pressure is most probably mediated centrally, as suggested originally by Chai and Wang[5], and not by some vasodilator mechanism[6,7]. The observed effects of diazepam were independent of any action that this agent may have had upon the growth pattern of the experimental animal (figure, B).

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Structure and antiarrhythmic activity of three ketophosphonium salts[1]

J. P. Hénichart and R. Houssin

Unité Inserm No. 16, Place de Verdun, F-59045 Lille Cedex (France), and Laboratoire de Chimie de Synthèse des Médicaments, Faculté de Pharmacie, F-59045 Lille (France), 28 July 1980

Summary. Three alicyclic organic phosphonium salts with an oxo and a morpholino group were found to exhibit a substantial antiarrhythmic action. The different activity levels were tentatively related to the structural modifications that they could induce in the cardiac cell membrane.

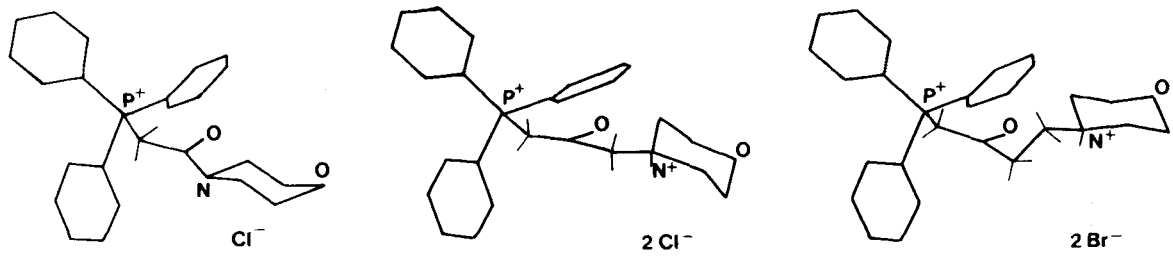
The 2 broad classes of agents used to correct arrhythmias[2,3] are specific drugs. β-adrenergic blockers such as propranolol, and non-specific ones such as quinidine which act as membrane-stabilizing agents[4]. Attempts to correlate the mode of action of 'quinidine-like' drugs with their molecular size revealed no simple relationships[5,6] when one considers the variety of their chemical classes.

However, the molecular structure of most non specific antiarrhythmic drugs consists of an aromatic portion connected with a basic amino group by way of an ester, ether or amide group. We present here the antiarrhythmic activity of 3 compounds possessing such structural requirements but where the ammonium electropositive site has been replaced by a phosphonium group, and we report their geometric characteristics to delineate possible structure-activity relationships (figure 1).

Material and methods. Synthesis of ketophosphonium salts. (2-oxo-2-morpholino) ethyltriphenylphosphonium chloride (OMETP) was prepared by treatment of chloroacetyl chloride with morpholine in 1,2-dichloroethane at -10 °C and further condensation of the thus-formed N-chloroacetylmorpholine (b.p. 106 °C/0.01 mm) with triphenylphosphine in dry benzene, refluxing under nitrogen for 5 h. The isolated precipitate was recrystallized from isopropanol (m.p. 225 °C).

(2-oxo-3-morpholino) propyltriphenylphosphonium chloride hydrochloride (OMPTP) was obtained by reacting (2-oxo-3-chloropropyltriphenylphosphonium chloride[7] with morpholine hydrochloride in the presence of sodium in ethanol under reflux for 3 h. Sodium chloride was filtered, ethanolic hydrochloric acid added to the filtrate and solvent evaporated. The crude product was recrystallized from ethanol (m.p. 165 °C).

(2-oxo-4-morpholino)butyltriphenylphosphonium bromide hydrobromide (OMBTP) was synthesized in anhydrous chloroform, by refluxing under nitrogen for 12 h, from



OMEPT OMPTP OMBTP

Fig. 1. Perspective drawn views of the 3 antiarrhythmic ketophosphonium salts.

Compounds	PMR data (δppm, $CDCl_3$) P–CH_2–CO	IR ν (cm^{-1})	Interatomic distance P^+–N (Å)	LD_{50} (mg/kg)	Doses (mg/kg)	Lethal dose of aconitine (µg/kg)	Protection (%)
Aconitine						423 ± 9	-
OMETP	5.71 (d, 2H, J_{P-H} = 13 Hz)	1630 (C=O) (KBr)	3.91	220 ± 3	80	571 ± 25 ($p \leq 0.001$)	40
OMPTP	5.15 (d, 2H, J_{P-H} = 15 Hz)	1730 (C=O) (KBr)	5.51	210 ± 5	80	590 ± 26 ($p \leq 0.001$)	35
OMBTP	Ketone: 6.10 (d, 2H, J_{P-H} = 12 Hz) Enol: 4.25 (d, 1H, J_{P-H} = 19.5 Hz) 12.80 (s, 1H, OH)	1720 (C=O) (KBr) 1615 (C=C) ($CHCl_3$) 3400 (O–H) ($CHCl_3$)	6.48	107 ± 3	40	545 ± 32 ($p \leq 0.01$)	29
Quinidine				200	100	493 ± 20 ($p \leq 0.01$)	16

triphenylphosphine and 1-bromo-4-morpholino-2-butanone hydrobromide (obtained by acid-catalyzed bromation of 4-morpholino-2-butanone[8]). The solvent was evaporated, the residue triturated with anhydrous ethanol-ether mixture and the resulting solid recrystallized from ethanol (m.p. 209 °C).

Structural determination. IR-spectra were taken on a double-beam Perkin-Elmer 177 spectrometer and ^1H NMR spectra were recorded on a JEOL JNM-MH-60 instrument. Accurate cell parameters were determined on a Philips PW 1100 diffractometer using MoK_α radiation. The structures were solved by direct methods using the Multan program[9]. The atoms were normally located by direct and difference Fourier maps except for some hydrogen atoms.

Pharmacological evaluation. The acute toxicity of the 3 compounds in mice following i.p. administration was evaluated by the Miller-Tainter method[10].

Antiarrhythmic potencies were assessed according to the method of Dadkar and Bhattacharya[11] applied to Swiss mice of either sex and 22-28 g b.wt. A slow perfusion (0.2 ml/min) of a 10 µ g/ml of aconitine into a caudal vein was performed and an electrocardiogram recording was taken. The aconitine doses to obtain cardiac arrest are noted. Tested drugs were administered by i.p. injections, 30 min before the beginning of the perfusion. Quinidine was employed as the standard.

Results and discussion. All the compounds showed a significant antiarrhythmic activity. In mice, they were found to be more efficient than quinidine against aconitine-induced arrhythmia. Nevertheless, the results clearly showed that the activity increased as OMBTP < OMPTP < OMETP and the relation between the observed change in their antiarrhythmic potency and their conformational differences was discussed.

It has been suggested that nonspecific antiarrhythmic agents appear to act directly on the myocardial cell membrane, producing a cardiodepressant effect[12]. This theory depends upon the ability of the drugs to bind at the cardiac membrane inducing a change in its conformation. In this model, OMETP, OMPTP and OMBTP may alter the structure of the membrane by hydrophobic interaction between the nonpolar tails of the phospholipids and the triphenyl moiety and by hydrogen bonding between the polar heads of phospholipids and the carbonyl groups. On the other hand, they can bind at the polypeptidic outer surface by the morpholinic portion (figure 2). The formation of such complexes could explain, to some extent, the blocking of the protein sodium channels by membrane expansion. Likewise, the charged tertiary amines could displace calcium ions from the membrane, competing with it for negatively charged binding sites. Thus, the 2 effects could explain the blockade of the ionic conductance necessary for action potential production.

By this reasoning, then, the structural changes due to OMBTP, OMPTP, OMETP could be factors influencing the membrane expansion and, therefore, the antiarrhythmic potency.

The conformations of the 3 compounds did not exhibit significant differences. The structural analysis in the solid state indicated that phosphonium and ketone groups are

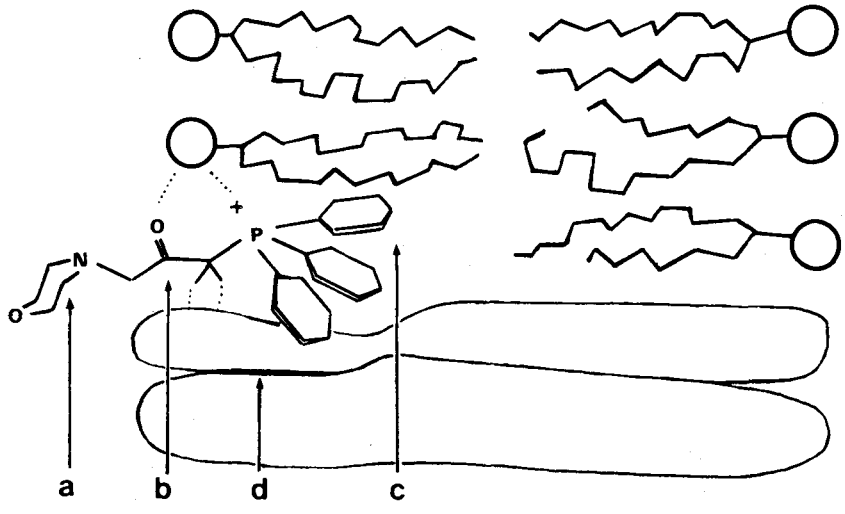


Fig. 2. Highly schematic illustration depicting possible physical membrane occupancy of OMETP, OMPTP and OMBTP: binding of amino group to the polypeptidic chain of outer surface (a), interaction with polar heads of phospholipids (b), hydrophobic repulsion of nonpolar tails of phospholipids (c) and distortion of protein resulting in blocking of sodium channel (d).

gauche with respect to the C_1-C_2 bond and the C=O group did not deviate far from the P-C_1-C_2 plane. However, spectral data in the solid state and in solution (see PMR results) show that OMBTP was a mixture of the keto and enol species. The perturbation of the electron density in this area could account for the loss of ability to form hydrogen bond and therefore could explain the decrease in antiarrhythmic potency. In the above hypothesis for the mechanism of action of the 3 molecules, the interatomic distances P^+-N may also play a deciding role and it is clear that the shorter molecule OMETP, the more potent compound, fits the membrane model well.

This distance separating the aromatic moiety from the nitrogen atom is in accordance with the geometrical data from crystal structures of well-known antiarrhythmic drugs with quinidine-like action such as quinidinium salts[13], diphenylhydantoin[14] and ajmaline[15].

This explanation is probably oversimplified but it provides a relatively straightforward rationale regarding the structure-activity relationship in the ketophosphonium salts series with the intention of attempting to map a 'receptor' for the antiarrhythmic agents.

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Acceleration of nerve regeneration by gangliosides estimated by the somatosensory evoked potentials (SEP)

F. Norido, R. Canella and F. Aporti[1]

FIDIA Research Laboratories, Via Ponte della Fabbrica, 3a, I-35031 Abano Terme, Padua (Italy), 20 December 1979

Summary. Cortical potentials disappear after peripheral denervation of the somatosensory pathway and are again detectable after regeneration of the transected stump. The characteristic increase of the sensory threshold is significantly reduced by daily ganglioside administration.

Gangliosides are complex glycosphingolipids generally localized in the plasma membranes especially of nervous cells[2-7].

Their structure is characterized by the presence of ceramide (hydrophobic side) and of one or more sialic acid molecules (hydrophilic side)[8].

The effectiveness of ganglioside treatment in influencing the regeneration of peripheral nerves in pathologic conditions has been assessed by several researchers in experimental[9-12] and clinical[13,14] studies.

The present work reports the influence of ganglioside administration on the modifications of somatosensory evoked potentials (SEP) recordings induced by surgical interruption of the sensitive pathway.

The establishment of chronic electrodes in the specific sensitive cortical area allowed us to study nervous regeneration of sensitive fibres in a peripheral mixed nerve, mostly constituted of motor fibres.

Materials and methods. A group of male Sprague-Dawley rats (crl: COBS CD (SD) BR) weighing 200–250 g was routinely anesthetized with i.p. injection of Sodium Thiopental. A stainless stell screw-type electrode was inserted into each skull, overlying the cortical tail projection area. A reference electrode was placed in the nasal bone.

After a 1-week post-operative period, the SEP evoked by stimulation of the rat tail was recorded. The stimulating and recording system consisted of a Romagnoli Elettronica Digit 3T stimulator, a stimulus isolation unit, a Tektronix 5A22N amplifier and a Ote Biomedica Neuroaverager 1172, connected with a X-Y L800 Linseis plotter. Continuous brain wave activity (EEG) was recorded on a polygraph to evidence motor activity. The averaging period was 100 msec after stimulus.

Bipolar stimulating electrodes were longitudinally inserted under the tail tendons near the 2 ventral longitudinal nerves at 6 cm from the tail base. Series of 10 square waves of 0.1-msec pulse duration and a frequency of 1 every 5 sec were selected. The threshold was determined by using a variable intensity (0.5–2 mA) sufficient to elicit the potential.

Each animal was subjected to preliminary control recording and those showing insufficient response were rejected. Surgical denervation of tail ventral nerves was then performed at 1 cm from the tail base and the transected stumps of the nerves were joined in an end-to-end anastomosis.

After a recovery period of a least 7 days, rats were divided into treated and untreated groups. Treated animals received a daily i.p. injection of 50 mg/kg of a bovine brain cortex ganglioside mixture consisting of 32% of GM_1, 38% of GD_{1a}, 17% of GD_{1b} and 13% of GT_1 and with a N-acetyl neuraminic acid content of 30.5% w/w. The other animals received a daily i.p. injection of saline.

A group of rats with chronic cortical electrodes inserted was treated with gangliosides but not denervated, to constitute the control animals.

Results. In order to establish SEP threshold, electric stimuli of increasing intensity were applied and submaximal and maximal evoked responses recorded. The SEP threshold is defined by the lowest electric stimulation intensity necessary to elicit SEP. The threshold was not modified in unlesioned animals over the period of experimentation (table 1).

The effect of denervation and reinnervation on the SEP recording was determined in the following manner. At variable time intervals after nerve transection, corresponding to different regenerative periods (16, 22, 28, 43 days),