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)

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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),

Table 1. SEP threshold in unlesioned control animals

Days	0	16	22	28	43
Stimulation intensity (mA)	1.8±0.122	1.7±0.122	1.7±0.122	1.8±0.122	1.5±0.2

Values represent means ± SE.

Table 2. Sensory threshold shift (mA) after denervation in treated and untreated rats

		Days after denervation 16	22	28	43
Increment of stimulation intensity (mA)	Treated (n=8)	1.687±0.230	1.625±0.206	1.500±0.250	1.312±0.230
	Untreated (n=8)	2.375±0.206	2.562±0.240	2.562±0.320	1.937±0.220
Δ (mA)		0.688	0.937	0.1062	0.625
Significance		$p<0.05$	$p>0.01$	$p=0.02$	not significant
% effect ± ES		29±11.5	36.6±10	41.4±12	32.3±14

Values represent means ± SE. Differences between the groups, significance and percentage effect are reported at bottom.

cortical potentials were recorded by applying electrical stimuli of increasing intensity.

During this period, amplitude and latencies of SEP appeared not to be significantly modified, while in all animals there was an increase of the current intensity required to elicit the potential (figure).

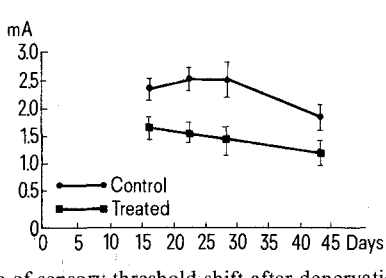
This increase reflected an enhancement of the threshold for the sensitive fibres, ensuing from nervous damage.

The rats treated with ganglioside mixture showed a threshold always significantly lower than that for untreated ones. After a nerve regeneration period of 16 days, a difference of 0.688 mA between the ganglioside-treated and untreated groups was found, corresponding to a reduction of 29% (table 2). After a regenerative period of 22 days, the difference between the 2 groups was of 0.937 mA corresponding to 36.6%. After 28 days, the difference of 1.062 mA corresponded to 41.4%. The last determination was then performed after 43 days and revealed a 0.625 mA difference with a percentage value of 32.3%.

A 2nd group of unlesioned animals was daily treated with ganglioside mixture and SEP also recorded after 16, 22, 28 and 43 days.

No significant differences were observed between recordings performed before and after ganglioside administration.

Discussion. The clinical application of SEP by means of a computer-averaging technique has been reported by several investigators[15,16]. In the present study the SEP threshold was used as an index of post-traumatic recovery and re-establishment of a classic sensitive pathway, i.e. ventral longitudinal nerves of the rat tail, spinal cord, thalamic nucleus and cerebral cortex. SEP disappears with nerve transection[17] and may be recorded after nervous regeneration, only following application of electric stimuli of higher intensity. During the process of reinnervation the sensitive threshold slowly moves toward normal values. The current intensity required to evoke SEP was always significantly lower in animals receiving ganglioside administration. After a regenerative period as long as 43 days, there is still a clear difference between the 2 groups, although with a reduced significance.



Time-course of sensory threshold shift after denervation. Ordinate, means of electrical stimulation intensity increments; abscissa, days after surgery. Vertical bars indicate standard errors.

The effectiveness of gangliosides in limiting the enhancement of the sensitive threshold detectable after denervation and reinnervation of the sensitive pathway may reflect an increased extent of functional reinnervation of sensitive peripheral fibres. In fact, ganglioside treatment of undernervated rats did not result in any substantial modification of SEP threshold, latency and amplitude values, indicating the denervated peripheral trunk as the site of the pharmacological action.

The ability of gangliosides to enhance the regenerative process and functional recovery after sensitive and motor nerve fibre degeneration and to stimulate the development of neuromuscular junctions in vitro[18] may reflect a common property of these constituents of the synaptosomal membranes.

Further application of autoradiographic, morphological and electrophysiological studies at cellular level will provide new helpful information on the specific role of gangliosides.

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