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THE ACTION AND ADSORPTION OF LOCAL ANESTHETIC ENANTIOMERS ON ERYTHROCYTE AND SYNAPTOSOME MEMBRANES

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SUMMARY

1. Two local anesthetics which were enantiomers of one another (spirosuccinimides RAC 109 I and II) protected human erythrocytes from hypotonic hemolysis at concentrations known to cause local anesthesia (0.35–1.6 mM). Both enantiomers produced 40 % anti hemolysis at 1.6 mM. The threshold concentration for anti-hemolysis with RAC 109 I extrapolated back to $1.15 \cdot 10^{-4}$ M, while that with RAC 109 II extrapolated to $1.92 \cdot 10^{-4}$ M.

2. The adsorptions of RAC 109 I and RAC 109 II to erythrocyte membranes were identical. The adsorptions of the two optical isomers to isolated synaptosomes (guinea pig brain) were also identical. All these adsorption isotherms were unaffected by the addition of 1 mM Ca^{2+}.

3. The membrane/buffer partition coefficients of the anesthetics on synaptosome membranes were identical to those on erythrocyte membranes.

4. The membrane/buffer partition coefficients were not constant, but varied inversely with the free concentration of the local anesthetic. Increasing the ionic strength also reduced the membrane/buffer partition coefficients, regardless of the free concentration.

5. At the exitability-blocking concentration of 2.5 mM, the concentration of local anesthetic in the membrane phase was about 0.02 mole drug per kg of dry membrane.

INTRODUCTION

A fundamental question in the molecular pharmacology of local anesthetics is whether there is stereospecificity or stereoselectivity in the action of enantiomers. Enantiomer differences in potency have been found with several local anesthetics[1–5] while with some enantiomer pairs equal potency was recorded[6,2]. ÅKERMAN *et al.*[7], for example, found that (+)-prilocaine was between 1.5- and 3-fold more potent than

Abbreviation: RAC 109, *N*-(γ-diethylaminopropyl)-1,2,3,4-tetrahydronaphthalin-1-spirosuccinimide.

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(–)-prilocaine in various tests for local anesthesia *in vivo*, but there was no difference *in vitro*.

It is impossible, however, to determine from these *in vivo* studies whether either of the enantiomers have a greater affinity for entities of the nerve cell membrane (and/or a greater efficacy). This is because differences in potency may be explained by unequal drug availability at the site of action which may be due to, *e.g.* differential rate of metabolism[8] or unequal rate of absorption[4,7,9].

Some of these interpretational difficulties are precluded by studying the blocking action of local anesthetics *in vitro*. It has been found by ÅKERMAN *et al.*[10–12] that the enantiomers of *N*-(γ-diethylaminopropyl)-1,2,3,4-tetrahydronaphthalin-1-spirosuccinimide (compound RAC 109) differ by about 2- to 8-fold in various tests for excitation–block potency, including isolated single nerve and muscle fibers. From these *in vitro* studies it appears that the succinimide enantiomers may differ in the 'intrinsic activity' or 'efficacy' in the membrane phase.

In order to measure the 'efficacy' experimentally, it is desirable to measure the amount of drug adsorption to the same cell membrane upon which the effect is studied. The use of nerve cells meets with technical difficulties in this case but the erythrocyte has been found convenient for this purpose since it can be readily isolated, and since all lipid-soluble anesthetics protect erythrocytes from osmotic hemolysis at concentrations similar or identical to those which anesthetize excitable membranes[13]. Anesthetic-induced protection of erythrocytes is associated with expansion of the cell membrane[14]. The 'efficacy' has been defined as the membrane effect of the anesthetic, divided by the number of anesthetic molecules in the membrane[15].

The present paper describes the adsorption onto erythrocyte membranes and the anti-hemolytic potencies of the enantiomers of a local anesthetic (compound RAC 109[16]) which have been found to differ in block of excitation both *in vivo* and *in vitro*[10,11]. The adsorption to synaptosome membranes was also studied.

METHODS AND MATERIALS

The anti-hemolytic actions of a pair of enantiomers

The enantiomers tested were those of *N*-(γ-diethylaminopropyl)-1,2,3,4-tetrahydronaphthalin-1-spirosuccinimide: RAC 109 I and RAC 109 II (refs. 10,11,16), as shown in Fig. 1. The compounds were used in the form of the hydrochlorides.

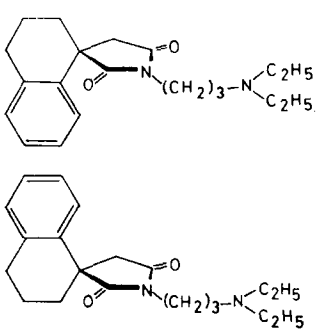

Fig. 1. Chemical structures of the enantiomers of *N*-(γ-diethylaminopropyl)-1,2,3,4-tetrahydronaphthalin-1-spirosuccinimide (RAC 109 I and RAC 109 II).

The method for testing the anti-hemolytic effects of local anesthetics has been described[13].

The adsorption of the enantiomers to membranes

Synaptosome membranes were prepared from guinea-pig brain according to the method of GRAY AND WHITTAKER[17]. Erythrocyte ghost membranes were prepared and the adsorption of [^{3}H]RAC 109 I (specific activity 7.3 μC/mg) and [^{3}H]RAC 109 II (specific activity 15.5 μC/mg) to these membranes was studied by the methods previously described[18,15]. The radioactive enantiomers were in the hydrochloride form.

The results for anti-hemolysis were analyzed in the form of ratios (% hemolysis with drug/% hemolysis without drug). The 95 % confidence limits and the Student t values were calculated by applying FIELLER's[19] theorem for the estimation of confidence limits of ratios.

RESULTS

The protective effect of the anesthetic enantiomers on human erythrocytes

The enantiomers of RAC 109 were approximately equipotent in protecting human erythrocytes from osmotic hemolysis. This is shown in Fig. 2, where the AH_{40} % for RAC 109 I was found to be $1.64 \cdot 10^{-3}$M, and for RAC 109 II to be

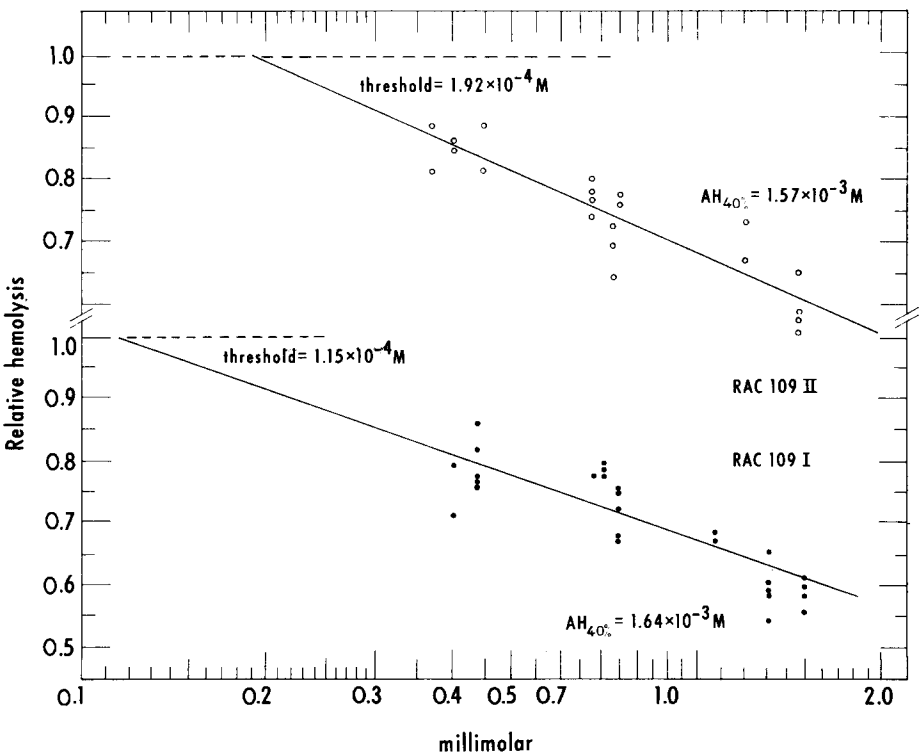

Fig. 2. The anti-hemolytic effects of the RAC 109 anesthetic enantiomers. There was no difference between RAC 109 I and RAC 109 II in the AH_{40} % values. The threshold concentrations (extrapolated) for anti-hemolysis, however, differed by 1.7-fold.

$1.57 \cdot 10^{-3}$M. It was more convenient to use the value for AH_{40} % rather than the AH_{50} % because these enantiomers caused hemolysis at concentrations higher than 3–4 mM. The two lines for the dose-response curves in Fig. 2 are not significantly different from parallel ($t = 1.17$; 49 d.f.). The threshold concentrations for anti-hemolysis with RAC 109 I and RAC 109 II extrapolated back to $1.15 \cdot 10^{-4}$ and $1.92 \cdot 10^{-4}$ M, respectively.

The adsorption of the radioactive enantiomers to membranes

The adsorption isotherms for RAC 109 I and RAC 109 II are presented in Fig. 3, where it can be seen that the two compounds have identical adsorption characteristics to synaptosomes and also to erythrocyte membranes.

The adsorption data were also analyzed in the form of partition coefficients, as shown in Fig. 4. It can be seen that the partition coefficients were inversely dependent on the free concentration of the drug.

Since 1 mM Ca^{2+} converts the permeability-increasing effect of chlorpromazine into a passive permeability-decreasing effect[20], it was important to study the effect of Ca^{2+} on adsorption. The addition of 1 mM Ca^{2+} had no effect on the adsorption of the optical isomers to either synaptosome or erythrocyte membranes (Fig. 3).

The membrane concentrations of the enantiomers and the effect of ionic strength on the adsorption of the compounds

In order to obtain the drug concentration in the membrane at the AH_{50} % or AH_{40} %, it is necessary to know the partition coefficient of the drug at different ionic

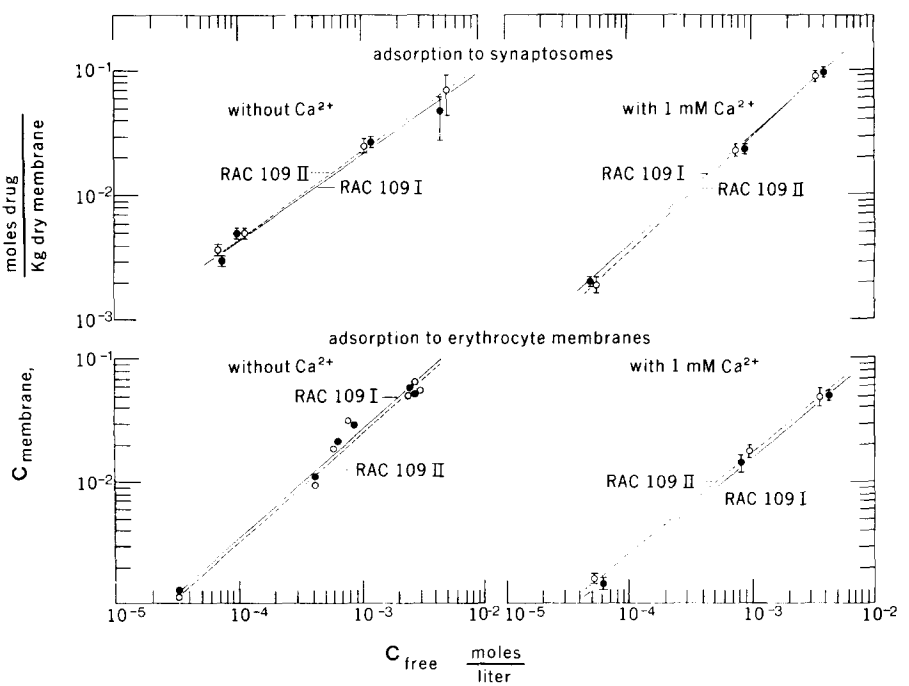

Fig. 3. The adsorption isotherms of the anesthetical optical isomers to synaptosome and erythrocyte membranes in both the absence and presence of 1 mM Ca^{2+}.

strengths, since the anti-hemolysis experiments were carried out at about 0.4 % NaCl and the adsorption isotherms were done in the presence of 15 mM Tris–HCl buffer only.

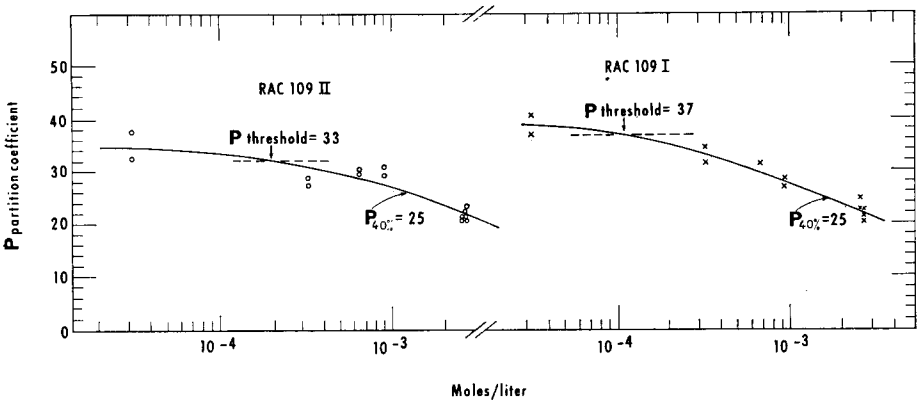

Fig. 4. The erythrocyte membrane/buffer partition coefficients for the anesthetic enantiomers of RAC 109. As with all anesthetic amines, the membrane/buffer partition coefficient varies inversely with the free anesthetic concentration.

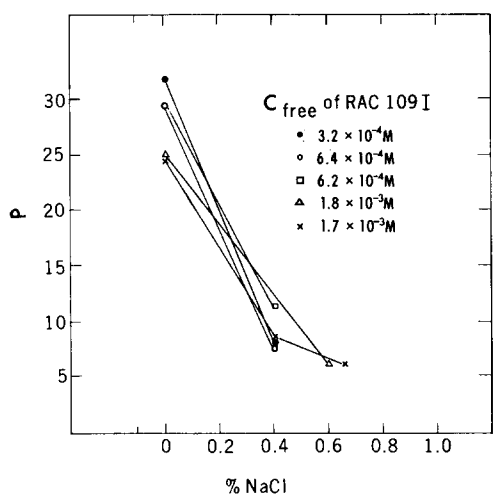

Fig. 5. The effect of ionic strength on the membrane/buffer partition coefficient of RAC 109 I. The partition coefficient varies inversely with the ionic strength, regardless of the free concentration of the anesthetic.

The effect of different NaCl concentrations on the partition coefficient of RAC 109 I is shown in Fig. 5. The partition coefficient decreased by 2–3-fold as the NaCl was increased. The AH_{50} % values for the two enantiomers extrapolated to 2.5 mM (from Fig. 2). At this extracellular concentration, the membrane concentration of the drug is equal to 2.5 mM · 8 = 0.02 mole/kg dry membrane, where 8 is the partition coefficient of the isomer at 0.4 % NaCl (Fig. 5). Virtually identical results were obtained for RAC 109 II (not plotted).

DISCUSSION

The results indicate: (1) that the enantiomers of RAC 109 have about equal effects on the human erythrocyte membrane, although the threshold concentrations for protection appear to differ by 1.7-fold; (2) that the enantiomers bind with equal affinity to synaptosomes and to erythrocyte ghost membranes; (3) that the membrane/buffer partition coefficients for both enantiomers were reduced by increased ionic strength and increased free drug concentrations; (4) and that at the extracellular concentration for AH_{50} % the concentration of both enantiomers in the membrane phase is approximately 0.02 mole/kg dry membrane.

For comparison of RAC 109 enantiomers acting on erythrocytes and on other tissues, a brief summary of the assay-systems upon which RAC 109 has been tried is presented in Table 1. Obviously the enantiomers exhibit stereoselective activity on electrically-induced activation of nerve and muscle but not on chemically-induced activation of muscle. The fundamental event in local anesthesia is an inhibition of the Na^+ influx which is associated with the generation of the action potential[21-24]. The evidence for this anesthetic inhibition of Na^+ influx had been based completely on electrical studies until recently[20] when it was shown that the local anesthetic,

TABLE I

A SUMMARY OF THE VARIOUS POTENCY RATIOS OF THE ENANTIOMERS OF COMPOUND RAC 109 IN DIFFERENT TEST SYSTEMS

The values are taken from the literature and the present results.

Pharmacological actions	*Approximate mean potency ratio*[10,11] *RAC 109 I/RAC 109 II*
(*A*) *Nerve in vivo*	
(1) Corneal anesthesia, duration	3.4
(2) Spinal anesthesia, duration	3.3
(3) Peridural anesthesia, duration of hind-limb paralysis	2.1
(4) Peridural anesthesia, duration of flexion reflex block	1.9
(5) Sciatic nerve block, duration of hind-limb paralysis	1.8
in vitro	
(6) Block of spontaneous electrical activity, crayfish ganglia	4.7
(7) Block of excitation, single nerve fibers	4.4
(8) Block of excitation (equilibrium), intact sciatic nerves	3.1
(9) Block of excitation (equilibrium), desheated sciatic nerves	1.9
(*B*) *Muscle in vivo*	
(10) Inhibition of ventricular tachycardia	2.0
in vitro	
(11) Inhibition of atrial excitability	4.0
(12) Elevation of threshold for exciting single extensor muscle fibers	3.0
(13) Reduction in rate of rise and amplitude of action potential of single extensor muscle fibers	1.0
(14) Anti-acetylcholine action, denervated single extensor muscle fibers	1.0
(15) Anti-acetylcholine, histamine and nicotine actions, ileum	1.0
(*C*) *Erythrocytes*	
(16) 50 % anti-hemolysis	1.0
(17) Threshold protection from hemolysis	1.7

chlorpromazine, reduced the passive influx of Na^+ into muscle and into erythrocytes.

The effects of the enantiomers on various threshold phenomena seems to be stereoselective. For example, the enantiomers exhibit different potencies in raising the threshold for stimulating muscle fibers (refs. 10,11; No. 11 and 12 in Table I). Similarly, the enantiomers differ by 1.7-fold in the extrapolated threshold concentrations at which erythrocyte protection begins. On the other hand, the enantiomers are equally potent in reducing the rate of rise of the action potential in muscle, in the same way as the enantiomers are about equi-potent in producing 40 % anti-hemolysis (No. 13 and 16 in Table I). The enantiomers were also about equally potent with regard to anti-hemolysis of rat erythrocytes (EDSTRÖM, personal communication).

Linked to the problem of threshold phenomena is the difficulty on considering the role of extracellular restrictions to drug equilibration. Since there are no extracellular structures adjacent to the erythrocyte plasma membrane, it is assumed that the drug comes into true equilibrium with the biophase immediately surrounding this membrane. Because of the extracellular barriers in contact with the plasma membrane of the neurone, it may be difficult to obtain a true equilibrium for the drug at the node of Ranvier. The extracellular barriers may act as non-specific binding sites to alter the steady-state concentration of drug in the biophase around the neurolemma; such sites might be stereoselective. However, the binding of the enantiomers of mepivacaine and bupivacaine to human plasma was not stereoselective[25]. The enantiomers of RAC 109 also bind equally to the epineural sheath of frog sciatic nerve (ÅKERMAN, unpublished results). In addition, a series of experiments (ÅKERMAN, unpublished results) gave the same uptake of the radioactive enantiomers by intact and desheathed nerves over various times and concentrations. Furthermore, the use of single nerve[10] and muscle[11] fibers revealed a significant difference in excitation block by the enantiomers. The ratio of the activities on single nerve fibers[10] were even greater than the differencies *in vivo*. Although, for example, it is known that the non-myelinated region of the node of Ranvier is approximately 80 % covered by the cytoplasmic processes of the interdigitating Schwann cells and is completely covered by a basement membrane[26,27], it does not seem particularly credible that these structures should act as stereoselective barriers to permeation. The findings do not seem to support the idea of unequal drug availability at the membrane level. It seems more conceivable that the enantiomers are taken up by excitable membranes with the same rate and to same the extent as they are taken up equally by the erythrocytes and synaptosomes in the present study.

It appears that where a threshold phenomenon is being tested, different potencies can be observed for local anesthetic enantiomers both in tissues which have one or more extracellular barriers (*e.g.* node of Ranvier) and in tissues which have little or no extracellular barriers (*e.g.* muscle, erythrocyte). Although Ca^{2+} did not affect the drug absorption (Fig. 3), preliminary findings suggest that Ca^{2+} may slightly alter the anti-hemolytic potencies of the enantiomers; it is known that Ca^{2+} and local anesthetics compete for the same membrane binding sites (see ref. 28 for references).

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