Strychnine and local anesthetics block ion channels activated by veratridine in neuroblastoma × glioma hybrid cells

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Benzocaine *Dibucaine* *Lidocaine* *Tetracaine* *Voltage-dependent sodium channel*

1. INTRODUCTION

Local anesthetics are drugs which block impulse conduction in nerves. They prevent the influx of Na^+ [1,2] by altering the increase in sodium permeability associated with the generation of action potentials. Despite the numerous efforts to elucidate the mechanism of local anesthetic action (citations in [3]), the literature on this subject is still confused. Controversies persist over the specific sites on which local anesthetics act. The difficulties are mainly due to the requirement of high concentrations so that the specificity can hardly be assessed. There are three main hypotheses to explain the blocking mechanism exerted by local anesthetics:

(i) Non-specific actions, such as perturbation of protein structure [4] or membrane expansion [5];
(ii) Changes in membrane surface charge [6,7];
(iii) Interaction with specific receptors [8,9].

Ion flux experiments using $^{22}Na^+$ have been proven a useful tool in studies of ionic channels (citations in [10]). The voltage-dependent Na^+ channel involved in the generation of action potential is also highly permeable to some other inorganic and organic cations, especially to guanidinium [11]. Thus [^{14}C]guanidinium fluxes have been used to study the Na^+ channel in neuroblastoma cells [12]. In mouse neuroblastoma × rat glioma hybrid cells [13,14] guanidinium uptake has been stimulated by veratridine and aconitine [15,16], two alkaloids activating the action potential Na^+ channel [17]. This system has been used to characterize pharmacologically the action potential Na^+ channel of the hybrid cells [15,16].These cells display many characteristics of neurons [14]. They are excitable upon electrical and chemical stimulation [14]. The inward current of their action potential can be carried either by Na^+ or by Ca^{2+} [18].

Here we report that various local anesthetic drugs affect the action potential Na^+ channel of the hybrid cells. The results show that dibucaine (IC_{50} = 0.1 mM), tetracaine (IC_{50} = 0.2 mM), procaine (IC_{50} = 0.8 mM), aminoacridine (IC_{50} = 0.1 mM) and strychnine (IC_{50} = 0.5–8 μM) inhibit the veratridine (or aconitine) -stimulated uptake of guanidinium in the hybrid cells. Such studies of ion flux can easily give information about the relative potencies of the various local anesthetic drugs, which would be far more difficult to achieve in electrophysiological studies.

2. MATERIALS AND METHODS

Culturing of cells has been described [14]. Neuroblastoma × glioma hybrid cells 108CC15 [13,14] or mouse neuroblastoma cells N1E-115 [19], initially seeded at 3.5 × 10^5 cells/plate (60 mm diam.) were grown for 3 days.

For measuring the uptake of guanidinium the growth medium was removed and 2 ml incubation medium added. The incubation medium consisted of 5.4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 2.0 mM Na_2HPO_4, 20 mM glucose, 20 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (adjusted to pH 7.4 with tris-(hydroxymethyl)-aminomethane), 145 mM NaCl or choline · Cl

Abbreviation: IC_{50}: half-maximal inhibitory concentration

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to keep the osmolarity at 320 mOsm/l. Together with 1 or 10 mM guanidinium • HCl (50–100 nCi ≙ 1.8–3.7 kBq) [^{14}C]guanidinium/ml (Amersham Buchler, Braunschweig) were added. The uptake has been shown to be linear with time for ≥ 20 min. Therefore, the incubation carried out at 37°C usually was stopped after 10 min by aspirating the medium and subsequently washing the cells 4 times with 3 ml ice-cold choline medium. The radioactivity retained in the cells was determined as in [15]. In parallel plates the protein content was measured using bovine serum albumin as a standard [20].

Tetracaine • HCl (4-(butylamino)benzoic acid 2-(dimethylamino)ethyl ester monohydrochloride), dibucaine • HCl (2-butoxy-*N*-[2-diethylamino) ethyl]-4-quinolinecarboxamide monohydrochloride), benzocaine (ethyl-*p*-aminobenzoate), veratridine, aconitine and scorpion toxin (from *Leiurus quinquestriatus*) were from Sigma, München; procaine (2-(diethylamino)ethyl *p*-aminobenzoate) from Hoechst AG, Frankfurt; lidocaine (2-diethylamino-*N*-(2,6-dimethylphenyl)acetamide) from Roth, Karlsruhe; 9-aminoacridine • HCl from EGA-Chemie, Steinheim; strychnine • sulfate from Merck, Darmstadt.

3. RESULTS

There is a great variation in the potency at which local anesthetics could inhibit the entry of guanidinium ions through the veratridine-activated ion channel in neuroblastoma × glioma hybrid cells (fig.1). Under the experimental conditions the veratridine-activated ion channel is most likely identical with the voltage-dependent sodium channel [15,16]. Half-maximal inhibition of guanidinium uptake (IC_{50}) was observed at 0.1 mM for both dibucaine and aminoacridine, at 0.2 mM tetracaine and at 0.8 mM procaine. The value for procaine was estimated on the basis of the assumption that the same maximal inhibition would be obtained as with the other compounds. Lidocaine and benzocaine had no significant effect at ≤ 1 mM. The local anesthetics similarly affected the basal uptake of guanidinium (legend to fig.1). Like the veratridine-stimulated guanidinium uptake, the veratridine-activated Li^+ uptake in the hybrid cells, characterized in [21], was inhibited by procaine (not shown).

Veratridine added with scorpion toxin stimulates the uptake of guanidinium more prominently than veratridine alone (fig.2). This enhanced stimulation probably resulting from a synergistic activation of sodium channels by veratridine and scorpion toxin [17], was inhibited by local anesthetics (fig.2). Dibucaine displayed the most potent inhibitory effect. For tetracaine the half-maximal inhibition was observed at 30 μM (not shown).

Similar experiments were done using neuroblastoma cells N1E-115 [19] (not shown). As in the hybrid cells, the veratridine-stimulated uptake of guanidinium was inhibited by the local anesthetics described here. The most potent substance was

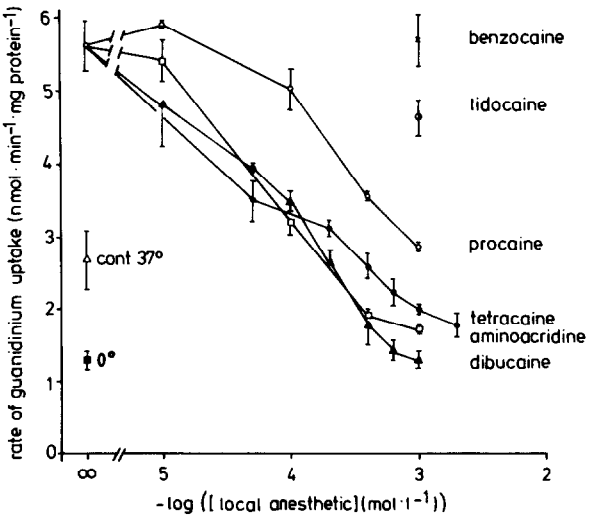

Fig.1. Rate of veratridine-activated guanidinium uptake in hybrid cells as a function of the concentration of various local anesthetics. The points designated with '0°' and 'cont 37°' represent the control values for uptake at 0°C and 37°C, respectively, in the absence of any drug. The results are representative for 4 similar expt. For the formulae of the local anesthetics see fig.2. The values for basal uptake of guanidinium at 37°C in the presence of 1 mM of the following local anesthetics were: tetracaine, 1.99 ± 0.14; lidocaine, 2.30 ± 0.11; procaine, 2.06 ± 0.34; dibucaine, 1.15 ± 0.11; and aminoacridine, 1.04 ± 0.11 nmol . mg protein $^{-1}$. min $^{-1}$. At 0.1 mM tetracaine, lidocaine and procaine had no significant effect on the basal uptake. The basal uptake in the absence of any drug is 2.7 ± 0.4 nmol . mg protein $^{-1}$. min $^{-1}$ (see point designated 'cont 37°').

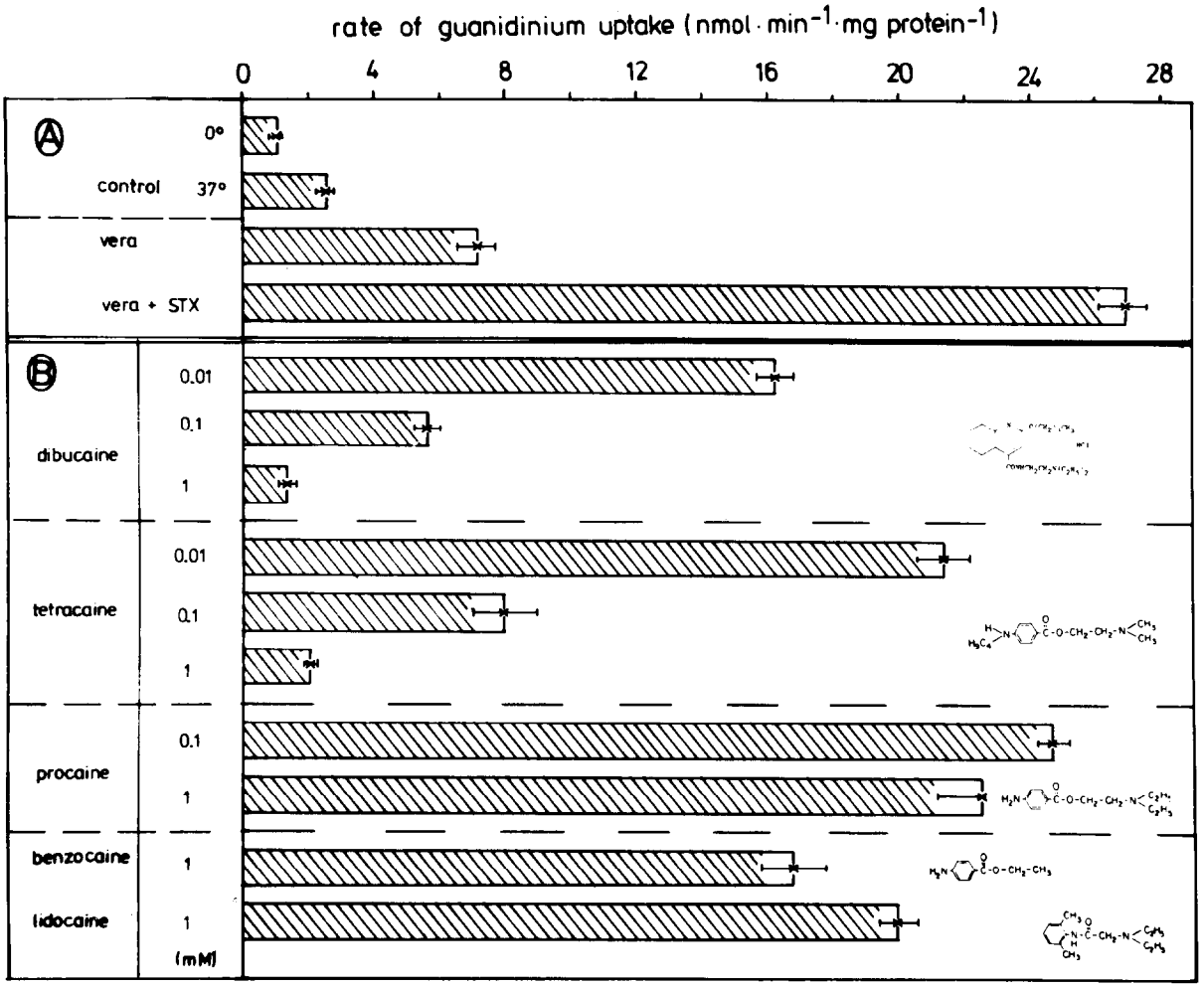

Fig.2. The effect of various local anesthetics on the guanidinium uptake by hybrid cells stimulated by veratridine plus scorpion toxin: (A) control values of guanidinium uptake at 0°C and 37°C and the stimulation by veratridine (vera, 0.2 mM) and by veratridine + scorpion toxin (STX, 50 μg/ml); (B) the influence of local anesthetics on the guanidinium uptake stimulated by veratridine + scorpion toxin. The results shown are representative for 2 expt with similar results.

dibucaine. Whereas tetracaine and procaine caused a small inhibition, lidocaine and benzocaine even at 1 mM showed no significant effect.

Strychnine blocked both the veratridine-stimulated and (to a moderate degree) the basal uptake of guanidinium in the hybrid cells (fig.3), the IC_{50}-values varying between 0.5–8 μM. Local anesthetics and strychnine inhibited the guanidinium uptake similarly to the inhibitions seen in fig.1 and 2, when the ion channel was activated by aconitine instead of veratridine (not shown).

4. DISCUSSION

Evidence has been presented here that in the hybrid and neuroblastoma cells the ion channel activated by veratridine is blocked by local anesthetics. The potency of the local anesthetics tested varied considerably. The aromatic amine benzocaine, which is essentially uncharged at physiological pH, displayed no effect. The tertiary aliphatic amines carrying a positive charge at physiological pH showed increasing potencies in the sequence:

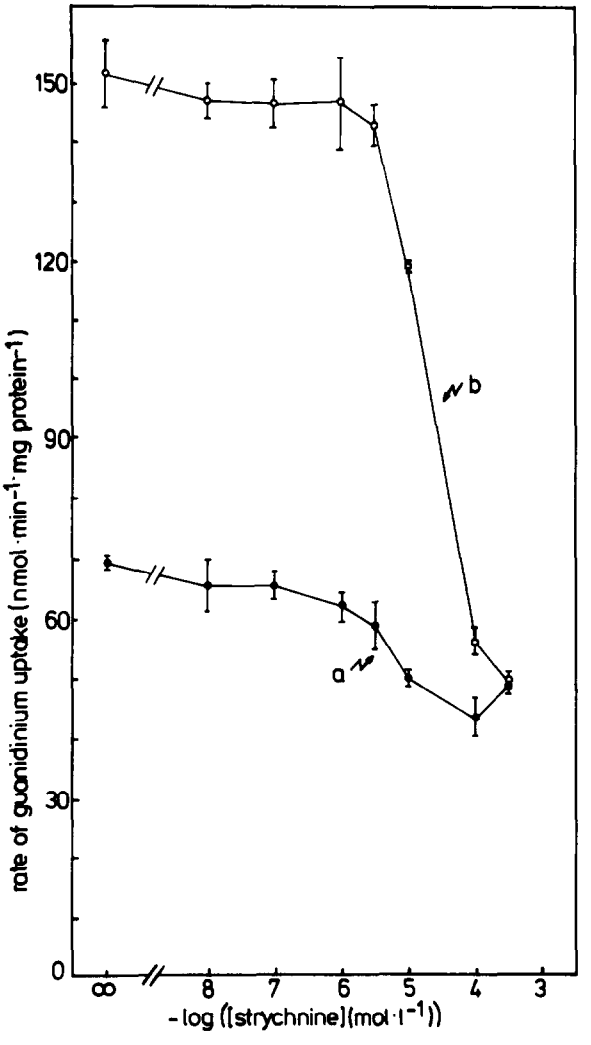

Fig.3. Basal (curve a) and veratridine-stimulated (curve b) uptake of guanidinium in hybrid cells as a function of the concentration of strychnine. Similar results were obtained in 3 other expt.

lidocaine < < procaine < tetracaine < dibucaine

The extremely lipid-soluble local anesthetic dibucaine (see in [3]) a tertiary aliphatic amine, turned out to be the most potent local anesthetic in the experiments described. However, upon stimulation with veratridine + scorpion toxin, lidocaine and benzocaine block guanidinium uptake more potently than procaine. This could be due to a different conformation of the ion channel, which is caused by the different modes of activation by veratridine or by veratridine + scorpion toxin.

In the hybrid cells strychnine inhibited the veratridine-induced activation. An analogous observation has been made in the frog node of Ranvier [22] and in the squid axon [23], where strychnine inhibited the sodium conductance in a way comparable to that for local anesthetics. The structural similarity of strychnine and lidocaine was indicated in [23]. We reported that propanolol blocked veratridine-activated guanidinium uptake in hybrid cells with IC_{50} 60μM [15,16] by a still unknown mechanism. Propanolol and other β-adrenoceptor blocking agents exert a substantial local anesthetic activity in several tissues (see [24]).

Models have been presented to describe the interaction of different local anesthetics with different sites on the sodium channel of neuroblastoma cells [25,26]. The hypothesis that local anesthetics act by 'chelating' Ca^{2+} has been doubted (citations in [3]). However, local anesthetics have been found in biochemical and in electrophysiological studies to interfere with Ca^{2+}–calmodulin-dependent processes as well as with Ca^{2+} channels [26,27].

The mechanism of action of local anesthetics and anesthetic-like drugs on the voltage-dependent Na^+ channel remains unclear. Further experiments yielding more information on the structure–activity relationship may help to distinguish between the possibilities that:

(i) Local anesthetic drugs interfere with the veratridine binding site;
(ii) Due to their charge, the drugs enter the ion channel and plug the pore, or they cause charge immobilization [28];
(iii) Since local anesthetics are readily lipid-soluble, they indirectly affect the opening of sodium channels.

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