Short communication

Modification of the Binding Sites for Local Anesthetics in Sodium Channels of Neuroblastoma Cells by Batrachotoxin and Aconitine

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Effects of the tertiary amines, procaine and lidocaine, its quaternary derivative QX—314, and neutral benzocaine, on Na currents in internally perfused neuroblastoma cells were studied using the suction-pipette voltage clamp technique (Kostyuk et al. 1975; Zubov et al. 1980). Neuroblastoma cells used were N18 A-1 and NTR-1 subclones derived from C1300 line (Konobasova et al. 1981) and Neuro 2a clone (American Type Culture Collection, 1981).

Changes in sodium currents (I_{Na}) caused by local anesthetics were examined both before and after modification of Na channels by batrachotoxin and aconitine. External administration of these toxins to the cell membrane was accompanied by repetitive (10 Hz) membrane stimulation to accelerate channel modification. The latter remained practically irreversible after the removal of batrachotoxin or aconitine from the solution. To avoid the effects of local anesthetics on the resting inactivation of Na channels, the holding potential, E_h, was set at -130 — -140 mV level.

The effects of local anesthetics on normal Na channels in neuroblastoma cells are qualitively similar to those in the nerve fibre (Khodorov 1978). Procaine, lidocaine (external administration) and QX—314 (internal administration) produce two phenomenologically different types of sodium current inhibitions: a steady block without conditioning stimulation, and a cumulative (use-dependent) block which develops during repetitive membrane pulsing. Benzocaine brings about a steady block only.

Modification of Na channels by batrachotoxin in neuroblastoma cells as well as in nerve fibres (Khodorov 1978; Zaborovskaya 1979) eliminates the cumulative inhibition of I_{Na} by amine local anesthetics. The cumulative effect was significantly reduced and in several experiments eliminated by aconitine, although aconitine modified Na channels undergo full incativation during membrane depolarization (Grishchenko et al. 1981).

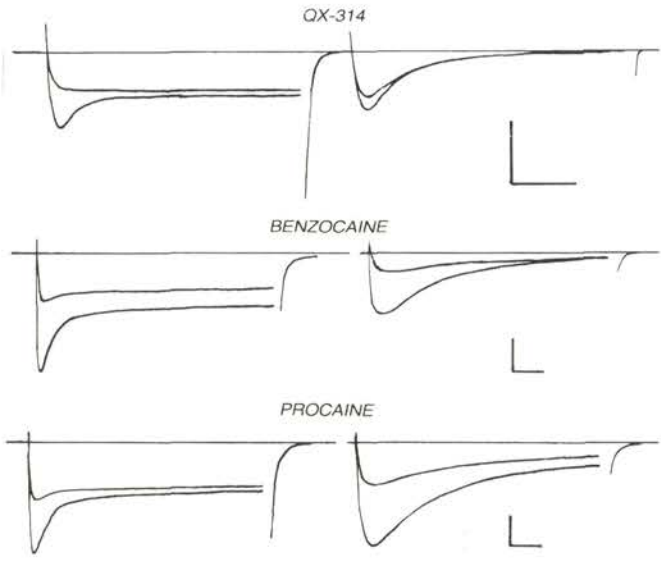

Fig. 1. Steady block produced by some local anesthetics in neuroblastoma cell sodium channels after their partial modification by 4×10^{-6} mol/l batrachotoxin (left column) and complete modification by 3×10^{-4} mol/l aconitine (right column). Note the presence of two current components in batrachotoxin-treated cells: The peak and the steady-state component, corresponding to unmodified and modified channel fractions, respectively.
QX—314 (1.7 mmol/l): applied internally. On the left: cell 332(80). Test voltage +20 mV. On the right: cell 339(80). Test voltage −10 mV. Clone NTR. Solutions contained (mmol/l): 140 KF, 5 Tris-HCl (internal); 132.5 NaCl, 10 $CaCl_2$, 7.5 tetraethylammonium (TEA)Cl, 5 Tris-HCl (external). Scales: 1 nA, 2 ms.
Benzocaine (1.0 mmol/l): applied externally. On the left: cell 95(82). Test voltage −30 mV. Scales: 2 nA, 2 ms. On the right: cell 98(82). Test voltage −50 mV. Scales: 1 nA, 1 ms. Clone Neuro 2a. Solutions contained (mmol/l): 130 KF, 10 TEACl, 5 Tris-HCl (internal); 140 NaCl, 2 $CaCl_2$, 5 Tris-HCl (external).
Procaine (1.0 mmol/l): applied externally. On the left: cell 95(82). Test voltage −30 mV. Scales: 2 nA, 2 ms. On the right: cell 99(82). Test voltage −50 mV. Scales: 1 nA, 1 ms. Clone Neuro 2a. Solutions as for benzocaine. Holding voltage (all records): −130 mV; pH of solutions 7.6; temperature approx. 20°C.

Table 1. Dissociation constants ($K_D \pm$ S.E., mmol/l) for normal and batrachotoxin or aconitine modified sodium channels in neuroblastoma cells. Figures in parentheses indicate the number of experiments.

Local anesthetic	Control	Modified by	
		Batrachotoxin	Aconitine
Benzocaine	0.68 ± 0.08(21)	3.42 ± 0.95(5)	0.35 ± 0.04(10)
Procaine	0.48 ± 0.13(6)	5.62 ± 0.12(2)	0.62 ± 0.08(7)
Lidocaine	1.21 ± 0.13(12)	5.66 ± 0.68(5)	9.10 ± 2.30(7)

The changes in the steady block of Na channels produced by procaine, lidocaine, QX—314 and benzocaine following batrachotoxin and aconitine treatment differed from each other, as shown in Fig. 1 and Table 1. Batrachotoxin led to a decrease in the sensitivity of Na channels to the steady blocking action of all the anesthetics used, while aconitine produced a diminution in this sensitivity to QX—314 and lidocaine only, leaving the effect of procaine obviously unchanged, while producing a significant raise in the blocking action of benzocaine.

The fact that batrachotoxin decreased the sensitivity of Na channels both to amine and neutral anesthetics means that the „protective effect" of batrachotoxin cannot be reduced to its influence on the hypothetical anionic site of this receptor (Khodorov 1981).

These effects are not identical to those observed in previous experiments with batrachotoxin and aconitine on nerve fibres. For instance, in the node of Ranvier batrachotoxin decreased the sensitivity of Na channels to amine drugs, it however did not affect the blocking action of benzocaine (Khodorov et al. 1977). At the same time, aconitine increased K_D for both procaine and benzocaine (Negulyaev and Nosyreva 1979). These differences may be due to differences in protein composition and/or those in the lipid environment of Na channels between the two membrane types.

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Received July 28, 1982/Accepted September 10, 1982