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INTERACTIONS OF LOCAL ANESTHETICS WITH NEURONAL 1,4-DIHYDROPYRIDINE BINDING SITES

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Abstract—A series of local anesthetics competed with 1,4-dihydropyridine [^{3}H]PN 200 110 binding to synaptosomes from guinea pig cerebral cortex synaptosomes. Dibucaine (10^{-3} M) increased the dissociation rate of bound [^{3}H]PN 200 110 consistent with an indirect, rather than competitive, interaction between local anesthetics and the 1,4-dihydropyridine site. The binding activities were compared to those previously available for local anesthetic competition with [^{3}H]batrachotoxinin benzoate binding at a Na^+ channel site. A linear correlation was observed between the two sets of activities with significantly higher activities being exhibited at the Na^+ channel site. This relationship is consistent with the significant structural homologies exhibited between Na^+ and Ca^{2+} channels.

The voltage-dependent Na^+ and Ca^{2+} channels may both be regarded as polyvalent drug receptors. These channels are sensitive to structurally diverse groups of drugs that interact at discrete, but allosterically linked, receptors to modulate channel function [reviewed in Refs. 1–3].

There are a number of important similarities between Na^+ and Ca^{2+} channels. These similarities include selective drug interactions with resting, open or inactivated channel states and the attendant therapeutic selectivity of action according to channel and tissue state [4, 5]. Thus, the Ca^{2+} channel antagonists, including the 1,4-dihydropyridine nifedipine and derived compounds, interact with high affinity at the inactivated state of the Ca^{2+} channel [6], and local anesthetics may similarly stabilize or access preferentially an inactivated state of the Na^+ channel [7].

These similarities likely derive, in part, from the considerable organizational and sequence homology that exists between Na^+ and Ca^{2+} channels [8, 9]. This suggests that there may be cross-over interactions in ligand specificity between these channel types [2] with potential therapeutic importance. Several examples of such interactions are known. Veratridine, a Na^+ channel activator, interacts with Ca^{2+} channels of neuroblastoma cells [10, 11], and the putatively Ca^{2+} channel selective 1,4-dihydropyridines also interact stereoselectively at the Na^+ channel, albeit with lower affinity, to demonstrate antagonist/activator properties [12, 13].

Our early work on [^{3}H]1,4-dihydropyridine binding at Ca^{2+} channels revealed a modest inhibitory action of lidocaine [11]. The activities of local anesthetics at the Na^+ channel measured by competition for [^{3}H]batrachotoxinin binding are available and correlate well with biological activities [14]. Accordingly, it seemed appropriate to examine a series of local anesthetics at the Ca^{2+} channel to provide a comparative assessment of activities.

MATERIALS AND METHODS

Tissue preparation. Synaptosomes were prepared from the cerebral cortex of male guinea pigs (weight 300–500 g, Buckberg Farms, Tomkins Cove, NY) according to previously published techniques [15, 16]. In brief, the cerebral cortex was homogenized in 10 vol./g wet weight of ice-cold 0.3 M sucrose, pH 7.0, with 10 passes in a glass–Teflon homogenizer driven by a TRI-R stirrer at setting 5. The homogenate was centrifuged at 1200 *g* for 10 min and the supernatant recentrifuged at 17,000 *g* for 20 min at 4°. The pellet was homogenized with a Pasteur pipette in 0.3 M sucrose, layered to 0.8 M sucrose solution, and centrifuged at 12,000 *g* for 20 min. The synaptosomes in 0.8 M sucrose were suspended in a buffer of the following composition [mM]: NaCl, 132; KCl, 5; $MgCl_2$, 1.3; $CaCl_2$, 1.2; glucose, 10; Tris base, 25, pH 7.4, and centrifuged at 14,000 *g* for 5 min at 4°. The synaptosomes were suspended in the same buffer at a protein concentration of 50–80 μg/5 mL binding assay volume. Cerebral cortex from Sprague–Dawley rats (Hilltop Farms, PA) was homogenized in 15 vol./g wet weight of ice-cold 50 mM Tris buffer, pH 7.2, with 10 passes in a glass–Teflon homogenizer at setting 5. The homogenate was centrifuged at 1,100 *g* for 20 min at 4°, the supernatant recentrifuged at 45,000 *g* for 45 min, and the pellet resuspended in 50 mM Tris buffer at a concentration of 180–220 μg/5 mL binding assay volume. Protein concentrations were determined by the method of Bradford [17] with bovine serum albumin as standard.

Radioligand binding. Synaptosomes were incubated with [^{3}H]PN 200110 (5.7×10^{-11} M) in the presence and absence of competing drugs for 90 min at 25°. Specific binding was defined by the presence of 10^{-6} M PN 200110 in a duplicate set of tubes. After the incubation period, the samples were filtered through GF/B filters with a Brandel cell

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Table 1. Inhibition of [^{3}H]PN 200 110 binding by local anesthetics in guinea pig brain cortex synaptosomes

Drug	IC_{50}* (M) [^{3}H]PN 200 110	n_H*	N	IC_{50}† (M) [^{3}H]Batrachotoxinin
Euprocin	$6.59 \pm 0.20 \times 10^{-5}$	2.03 ± 0.16	6	7.4×10^{-7}
Dibucaine	$2.38 \pm 0.20 \times 10^{-4}$	0.94 ± 0.04	6	1.4×10^{-6}
Tetracaine	$1.49 \pm 0.08 \times 10^{-3}$	1.67 ± 0.08	6	3.4×10^{-6}
Butacaine	$2.12 \pm 0.20 \times 10^{-3}$	0.91 ± 0.04	6	2.7×10^{-6}
Quinidine	$2.76 \pm 0.12 \times 10^{-3}$	1.80 ± 0.07	6	3.2×10^{-6}
Bupivacaine	$4.10 \pm 0.52 \times 10^{-3}$	1.23 ± 0.12	5	5.4×10^{-6}
Benzocaine	$4.67 \pm 0.51 \times 10^{-3}$	1.45 ± 0.14	7	9.1×10^{-4}
Lidocaine	$1.48 \pm 0.19 \times 10^{-2}$	1.45 ± 0.18	3	2.4×10^{-4}
Procaine	$1.10 \pm 0.11 \times 10^{-1}$	2.24 ± 0.19	3	1.1×10^{-4}
Cocaine	19.13 ± 3.77‡		5	4.9×10^{-5}

* Mean ± SEM.
† Data from McNeal *et al.* [14] for guinea pig brain synaptosomes.
‡ Percent inhibition at 10^{-2} M.

harvester (model M-24R, Brandel Instruments, Gaithersburg, MD) and washed twice with 5-mL aliquots of ice-cold buffer.

In dissociation experiments, membranes were incubated with 8.4×10^{-11} M [^{3}H]PN 200 110 for 120 min at 25°. After incubation, 10^{-5} M PN 200 110 with or without 10^{-3} M dibucaine was added and, at the indicated times, samples were filtered. Nonspecific binding was determined by the presence of 10^{-6} M PN 200 110. Filters were counted by liquid scintillation spectrometry at an efficiency of approximately 45%.

Data analysis. IC_{50} Values for local anesthetic inhibition of [^{3}H]PN 200 110 binding were determined from concentration–response curves using probit analysis [18]. Dissociation rate constants were determined using a non-linear curve fitting program [19]. The significance of differences of dissociation rates of [^{3}H]PN 200 110 in the absence and presence of dibucaine was determined using a grouped Student's *t*-test.

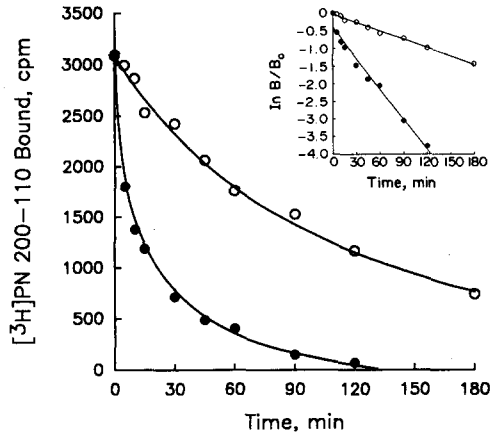

Fig. 1. Time course of dissociation of [^{3}H]PN 200 110 in the absence (○) and presence (●) of 10^{-3} M dibucaine in rat cerebral cortex membranes. Inset: semilogarithmic representation of the dissociation. Data are from one representative experiment out of four or five, each performed in duplicate.

RESULTS

Competition binding. [^{3}H]PN 200 110 binding in guinea pig cerebral cortex synaptosomes was saturable and of high affinity with K_D and B_{max} values of $3.0 \pm 0.7 \times 10^{-11}$ M and 469 ± 60 fmol/mg protein, respectively. These data are quite consistent with published values [reviewed in Ref. 20]. The local anesthetics inhibited specific [^{3}H]PN 200 110 binding (Table 1). Euprocine was the most active agent and cocaine the least active: Cocaine at 10^{-2} M produced an approximately 20% inhibition of binding. Limitations of solubility prevented higher concentrations of these agents from being employed. Ethanol was used as the solvent and control experiments were run to determine any effects of the vehicle alone on the binding of (+)-[^{3}H]PN 200 110 [11]. The pseudo-Hill (slope) coefficients were markedly greater than unity save for dibucaine, butacaine and bupivacaine. This likely indicates, as anticipated, interactions more complex than simple competition between local anesthetic and 1,4-dihydropyridine binding sites.

Effect of dibucaine on [^{3}H]*PN 200 110 binding.* The dissociation kinetics of [^{3}H]PN 200 110 in the absence and presence of dibucaine are shown in Fig. 1 for rat cerebral cortex membranes. A single exponential dissociation rate was observed in the absence of local anesthetics of $k_{-1} = 7.92 \pm 0.35 \times 10^{-3}$ min^{-1}. Dibucaine significantly ($P < 0.05$) increased the dissociation rate to $2.63 \pm 0.22 \times 10^{-2}$ min^{-1}.

DISCUSSION

As class I antiarrhythmics, the local anesthetics are known to bind to sites associated with the voltage-dependent Na^+ channel [*inter alia*, Refs. 21

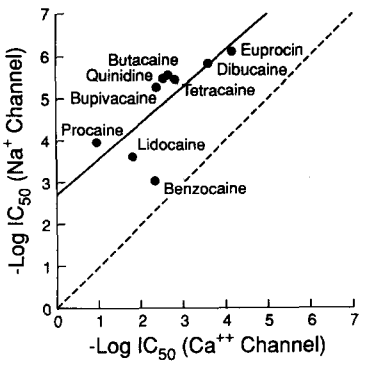

Fig. 2. Correlation between the inhibitory activities of a series of local anesthetics at sodium and calcium channels (data from Table 1). The solid line represents the regression described by the equation: log $1/IC_{50}[Na^+] = 0.86 \pm 0.30$ log $1/IC_{50}[Ca^{2+}] + 2.70 \pm 0.83$ ($N = 9$, $r = 0.73$, $P < 0.05$). The dashed line represents the line of equivalency.

and 22]. The ability of local anesthetics to interact allosterically with [^{3}H]batrachotoxinin A benzoate binding serves as a quantitative index of activity that correlates well with the known local anesthetic potencies of these agents [14]. Local anesthetics are known to interact also with voltage-dependent Ca^{2+} channels in both radioligand binding and electrophysiologic protocols. Lidocaine and procainamide exhibit a modest degree of interaction with [^{3}H]nitrendipine binding in intestinal smooth muscle [11]. A more detailed examination in brain and heart revealed generally similar potencies, but different sensitivities to mono- and divalent cations [23]. In guinea pig ventricle, bupivacaine and lidocaine completely block the calcium-mediated action potentials at concentrations of 10^{-4} and 5×10^{-4} M, respectively [24]. This study confirmed the inhibition of [^{3}H]PN 200 110 binding by local anesthetics (Table 1). Because dibucaine, as a representative local anesthetic, increased the dissociation rate of [^{3}H]PN 200 110 from its binding sites, these interactions are likely to be allosteric rather than simply competitive.

The present study compares, for a series of local anesthetics, their activities as inhibitors of (+)-[^{3}H]PN 200 110 binding and [^{3}H]batrachotoxinin A benzoate binding in guinea pig cerebral cortex synaptosomes. Without exception, these agents were more effective at Na^+ channels than at Ca^{2+} channels by factors of between 100- and 1000-fold (Table 1). However, a correlation did exist between Ca^{2+} channel and Na^+ channel activities (Fig. 2), although it covered a limited range of activities. Major deviations were provided by benzocaine which, relative to the charged anesthetics, was comparably active at both Na^+ and Ca^{2+} channel sites and by cocaine which was weakly active at Ca^{2+} channels even at a concentration of 10^{-2} M.

These data indicate that the clinically achieved concentrations of local anesthetics are unlikely to have major actions at the Ca^{2+} channel. However, the general parallelism of the structure–activity relationships depicted in Fig. 2 is consistent with similarities in binding site structure achieved through structural homology. The quantitative differences observed likely arise in part from these differences and in part from the channel-mediated state-dependent interactions. [^{3}H]Nitrendipine binds with higher affinity to inactivated states of the Ca^{2+} channel [6, 25] and [^{3}H]batrachotoxinin to an open state of the Na^+ channel [26]. Accordingly, anesthetic activities at the Na^+ and Ca^{2+} channels are being compared under different conditions [27]. Similar considerations of state-dependent interactions likely underlie the quantitative differences in activity of 1,4-dihydropyridine Ca^{2+} channel antagonists in smooth and cardiac muscle [3, 28].

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