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# Local Anesthetics Inhibit Substance P Binding and Evoked Increases in Intracellular $Ca^{2+}$

*Yue-Ming Li, Ph.D.,* Douglas E. Wingrove, M.D., Ph.D.,† H. Phon Too, Ph.D.,* Margarita Marnerakis, B.S.,‡ Evelyn R. Stimson, Ph.D.,§ Gary R. Strichartz, Ph.D.,‖ John E. Maggio, Ph.D.#*

***Background:*** **During spinal and epidural anesthesia, local anesthetics reach concentrations in cerebrospinal fluid and spinal cord tissues at which their actions may extend beyond the classic blockade of sodium channels. This study examines the effects of several clinical and experimental local anesthetics on the binding and actions of a peptide neurotransmitter, substance P, known to be important in nociceptive transmission in the dorsal horn.**

***Methods:*** **The binding of radiolabeled (Bolton-Hunter modified) substance P was studied in chick brain membranes in the presence of local anesthetics. The increase in intracellular calcium $[Ca^{2+}]_{in}$ evoked by substance P was measured by the fluorescent indicator fura-2 loaded in a murine cell line expressing substance P (NK1) receptors. Cells were preincubated with bupivacaine before and during the transient addition of substance P.**

***Results:*** **Both substance P binding and $Ca^{2+}$ increase were inhibited half-maximally by approximately 1 mM bupivacaine at *p*H 7.5, whereas tetracaine, lidocaine, and benzocaine were slightly less potent at inhibiting binding. Concentration-dependent substance P-binding studies showed that bupivacaine's inhibition was not competitive. Inhibition of substance P binding by bupivacaine increased with increasing *p*H, but the protonated species appears to have some inhibitory activity, and quaternary lidocaine also inhibited binding. There was no stereoselectivity to the binding inhibition.**

***Conclusions:*** **Because millimolar concentrations of local anesthetics are within the range measured in spinal cord during intrathecal and epidural procedures, these results are consistent with a direct action of local anesthetics on tachykinin-mediated neurotransmission during regional anesthesia. (Key words: Anesthetics, local: bupivacaine. Ions, calcium: intracellular. Neurotransmitters, substance P: tachykinins. Receptors, substance P: NK1.)**

LOCAL anesthetics are used to provide regional and local relief of pain from surgical procedures or accidental trauma and to relieve chronic pain syndromes. Anesthetic agents may be injected near a peripheral nerve, around the spinal cord (intrathecally or epidurally), applied topically on the skin or mucosal surfaces, or injected intravenously at low doses.[1] All of these routes are usually effective in providing analgesia.

Traditionally, local anesthetics are thought to act through the inhibition of voltage-gated $Na^+$ channels by preventing neuronal impulse propagation or generation.[2,3] However, at the doses used clinically, *e.g.*, 0.5–2% lidocaine, corresponding to undiluted concentrations of 18–75 mM and final tissue concentrations of 1–4 mM,[4,5] functions other than impulse propagation often are affected by local anesthetics.[6] Among these functions are those mediated by a broad variety of membrane-intrinsic proteins, including catalytic enzymes, such as phospholipases and protein kinases, and members of the superfamily of receptors having seven putative transmembrane domains, such as the muscarinic cholinergic receptors, β-adrenergic receptors, and as shown in the current study, the NK1 (substance P) tachykinin receptor.

The tachykinin peptides are important transmitters/modulators in nociception.[7,8] Tachykinin receptors are found in the central synapses of the dorsal horn and brain, in peripheral cutaneous tissue,[9,10] and in other areas. Substance P, the archetypal tachykinin, is an undecapeptide found, among other places, in both the central and peripheral terminals of primary afferent

* Research Fellow in Biological Chemistry and Molecular Pharmacology.

† Instructor in Anesthesia.

‡ Research Assistant in Biological Chemistry and Molecular Pharmacology.

§ Research Associate in Biological Chemistry and Molecular Pharmacology.

‖ Professor of Anaesthesia (Pharmacology).

# Associate Professor of Biological Chemistry and Molecular Pharmacology.

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Address correspondence to Dr. Maggio: Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts 02115.



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(likely nociceptive) neurons.[11] Substance P binds to its NK1 receptor to produce or modulate pain and inflammation. Thus, both the peptide and its receptor are appropriately located in the dorsal roots and spinal cord, respectively, and noxious stimuli enhance substance P release from C-fibers. Substance P depolarizes dorsal horn neurons in a manner similar to the depolarization evoked by electrical stimulation of C-fibers, and both responses are blocked by substance P receptor antagonists. Substance P is hyperalgesic, and substance P antagonists are analgesic. Animals treated neonatally with capsaicin, and therefore having many fewer sensory substance P-ergic neurons and much less substance P in dorsal root ganglia and dorsal spinal cord than control animals, show increased pain thresholds.[8]

To the extent that local anesthetics prevent the binding of substance P to its receptor or inhibit any of the subsequent responses evoked by the activated receptor, pain responses will be ablated or diminished by actions independent of sodium channel inhibition. The purpose of this study was to examine substance P binding and cellular response to substance P for their sensitivity to local anesthetics.

## Methods and Materials

### *Receptor-binding Assays*

Radiolabeled substance P (labeled with $^{125}$I-Bolton Hunter reagent at Lysine-3; $^{125}$I-BHSP) was synthesized as previously described[12] and purified by reverse-phase HPLC to a specific activity of 2,000 Ci/mmol. Preparation of chick brain membranes and binding assays were performed as described by Too and Hanley.[13] Briefly, membranes were incubated with labeled BHSP (0.1 nM) and protease inhibitors in the presence or absence of unlabeled peptide or local anesthetic for 20 min before separation of membrane-bound from free ligand by filtration. Local anesthetics and unlabeled peptides were added, as solutions in dimethylsulfoxide, to the assay mixture at the start of incubation. Nonspecific binding (*i.e.,* complete inhibition of specific binding) is defined as binding in the presence of 10 μM unlabeled substance P. The final concentration of dimethylsulfoxide was less than 3% vol/vol; this concentration has no effect on the assays described. All binding experiments were conducted at room temperature (approximately 22°C).

### *Cell Culture*

The murine cell line P388D$_1$[14,15] was purchased from the American Type Culture Collection and has been maintained in our laboratory in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Cells were passaged the day before use and were of a similar density at the time of the experiments. The cells were not cultured in the presence of local anesthetics. At the concentrations of bupivacaine used in this study, the size and appearance of the cells was not affected by bupivacaine.

### *Calcium Measurements*

P388D$_1$ cells were cultured on 12 mm-diameter round glass coverslips that had been pretreated with laminin and were used within 24 h after plating. Dye loading was achieved by exposing the cells to fura-2 acetoxymethyl ester at a concentration of 8 μM for 30 min at room temperature. The cells were washed with isotonic NaCl solutions containing 2% bovine serum albumin and were kept on ice until used. Experiments were performed using a standard saline buffer with the following components: NaCl 120 mM, KCl 4.2 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.0 mM, $Na_2HPO_4$ 1.0 mM, glucose 12 mM, and HEPES 10 mM, *p*H 7.40.

Fluorescence measurements were made using a Nikon microscope optically linked to a PTI Deltascan instrument that produces dual excitation at 340 and 380 nm. Emitted light was collected after passing through a 510-nm bandpass filter. A 40X Nikon fluor objective was used, and the field was limited to about 15–20 cells for data collection. All measurements were taken on cells at similar density. The presence of local anesthetics at the concentrations employed had no detectable effects on cell size or density.

Calcium concentrations were calculated using the ratio of fluorescence when excited at 340 nm to that at 380 nm (R = F340:F380) according to the method described by Grynkiewicz *et al.*[16] The $R_{min}$ value (R at zero free calcium) was obtained by exposing the fura-2-loaded cells to a calcium-free buffer similar to that described above except that it contained 10 mM EGTA to chelate free $Ca^{2+}$ and 10 μM Br-A23187, a calcium ionophore, to release $Ca^{2+}$ from within the cells. After the zero $Ca^{2+}$ signal was obtained at equilibrium, a $CaCl_2$ plus Tris buffer was added to yield 5 mM free calcium at *p*H 7.40, and the $R_{max}$ value (R when dye was saturated with calcium) was determined. This procedure yielded an $R_{min}$ of 0.52, an $R_{max}$ of 3.43, and an $S_{f2}/S_{b2}$ of 3.06. A $K_d$ for fura-2 and $Ca^{2+}$ of 224 nM was assumed. The background fluorescence was subtracted from all measurements before calculations.



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**Table 1. Inhibition of $^{125}$I-Bolton-Hunter Substance P Binding to Chick Membrane by Local Anesthetics at *p*H 7.5**

| Local Anesthetic (1 mM) | Inhibition of BHSP Binding (% control) |
|---|---|
| Bupivacaine | 49.3 ± 2.3 |
| Tetracaine | 35.7 ± 10.7* |
| Benzocaine | 22.7 ± 4.0*† |
| QX314 | 22.1 ± 1.5*† |
| Lidocaine | 18.7 ± 0.3*† |

Values are mean ± SE of at least three experiments.

* Significantly different from bupivacaine ($P < 0.01$).

† Significantly different from tetracaine ($P < 0.05$).

### *Statistical Methods*

Results are reported as mean ± SE of multiple experiments. Statistical significance of the differences of means are calculated using Student's two-tailed *t* test.

## Results

The specific binding of radiolabeled substance P to chick brain membranes at *p*H 7.5 was significantly inhibited by various local anesthetics at concentrations of 1 mM (table 1). The rank order of inhibition at 1 mM local anesthetic was bupivacaine > tetracaine > benzocaine ≈ QX314 (a cationic quaternary lidocaine homolog) ≈ lidocaine.

Inhibition by local anesthetics was concentration-dependent (fig. 1), with significant effects in the range of concentrations achieved clinically (see below).

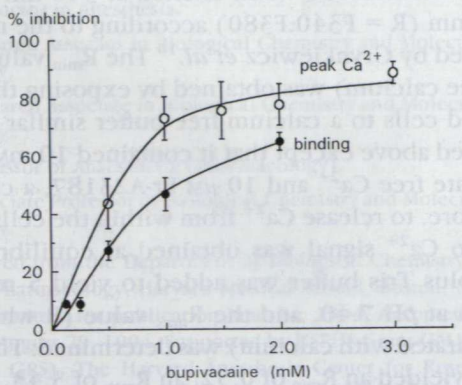


**Fig. 1. Inhibition of peak $Ca^{2+}$ response to substance P in P388D$_1$ cells (open circles) and inhibition of $^{125}$I-BHSP binding to chick brain membranes (closed circles) by bupivacaine. Each point represents mean ± SEM of at least three experiments.**

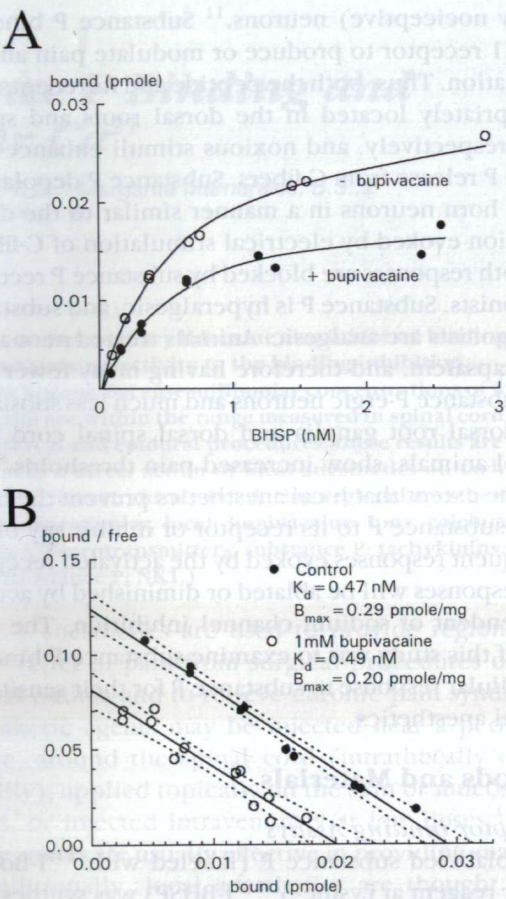


**Fig. 2. Binding of $^{125}$I-BHSP to chick brain membranes in the presence (closed circles) and absence (open circles) of 1 mM bupivacaine as saturation isotherms (*A*) and Scatchard plots (*B*). Results from two experiments, each done in duplicate. Hill plots of the data presented in *A* show slopes not significantly different from unity ($n_H$ = 1.08 and 0.97 in the absence and presence of bupivacaine, respectively). Dashed lines in *B* indicate 95% confidence intervals.**

Enantiomers of a chiral bupivacaine carbamide homolog, HS37, inhibited BHSP binding equally (49% for the R- and 46% for the S-enantiomer, both at 2 mM).

Further analysis established that the inhibition of BHSP binding by bupivacaine was not competitive, because the apparent $B_{max}$ was lower, but $K_d$ was unchanged in the presence of bupivacaine (fig. 2). Hill slopes were unaffected by the presence of this local anesthetic (data not shown).

The degree of inhibition of BHSP binding to membranes by bupivacaine was a function of *p*H (fig. 3). Thus, inhibition by bupivacaine increased with alkalinization relative to *p*H-matched controls. Local an-



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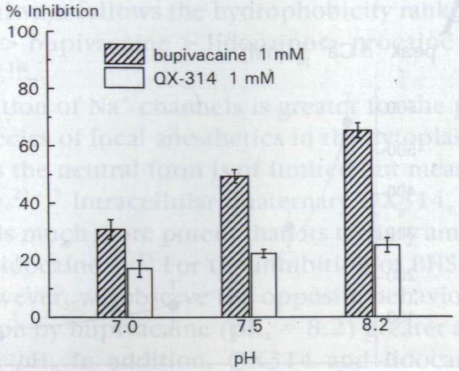


**Fig. 3. Inhibition of $^{125}$I-BHSP binding to chick brain membranes by 1 mM bupivacaine (hatched bars) or QX314 (open bars) at three *p*H values, relative to *p*H-matched control binding. Each bar represents mean ± SEM of three experiments. Inhibition by 1 mM bupivacaine at *p*H 7.0 and 8.2 differ significantly ($P < 0.02$) from inhibition at *p*H 7.5. Inhibition by 1 mM QX314 does not differ significantly ($P > 0.2$) among the *p*H values tested.**

esthetics of fixed charge, benzocaine (uncharged, data not shown), and QX314 inhibited BHSP binding equally at every *p*H (fig. 3).

The murine cell line P388D$_1$ expresses substance P (NK1) receptors and displays a transient increase in cytoplasmic $Ca^{2+}$ upon exposure to substance P.[15] Two components of the transient (fig. 4) are detected: a steeply increasing, early "peak" component followed by a lower, longer-lasting plateau. Both components

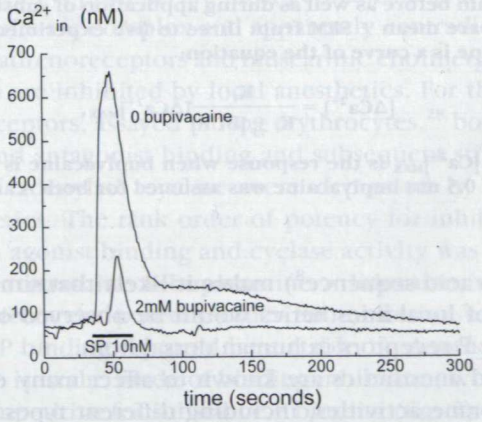


**Fig. 4. Time course of the change in $[Ca^{2+}]_{in}$ measured with fura-2 in P388D$_1$ cells. Substance P ($10^{-8}$ M) was perfused over the cells during the time of the heavy horizontal bar. This typical result from a single run over an optical field containing 12–18 cells shows the peak and plateau phases of increased $[Ca^{2+}]_{in}$ in the absence and presence of 2 mM bupivacaine. Experiments were conducted at room temperature (approximately 24°C).**

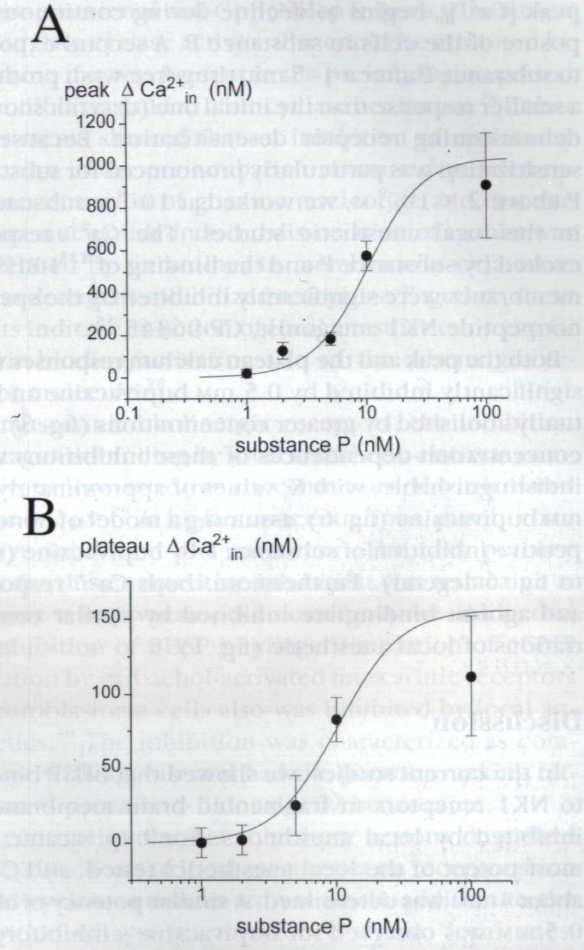


**Fig. 5. Change in $[Ca^{2+}]_{in}$ *versus* substance P in superfused P388D$_1$ cells. (*A*) Height of the peak response and (*B*) value during the plateau phase (see fig. 4). Each data point is the mean ± SEM from four to six separate measurements. Ordinates in figures 5 and 6 are the changes above resting $[Ca^{2+}]_{in}$. The average resting $[Ca^{2+}]_{in}$ was 83 ± 12 nM (mean ± SEM). The fitted curves are modified rectangular parabolas of the form:**

$$y = \frac{aX^b}{(c + X_b)},$$

**where $y$ = calcium response, $X$ = substance P, and $a$, $b$, and $c$ are empirical constants. For the curve in *A*, $a = 1{,}040$, $b = 1.9$, and $c = 79$; for *B*, $a = 165$, $b = 1.9$, and $c = 79$. In *B*, the scatter at substance P concentrations above 100 nM was large. All these data were used, however, for the curve fitting to arrive at a more accurate estimate of the level of $[Ca^{2+}]_{in}$ produced by saturating substance P.**

increase with increasing substance P, being half-maximally activated at 5–10 nM substance P (fig. 5). (At $10^{-6}$ M $Ca^{2+}$, fura-2 fluorescence is a nonlinear measure of intracellular $Ca^{2+}$; therefore, the highest value in figure 5A carries little quantitative weight.) Stimulated



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peak $[Ca^{2+}]_{in}$ begins to decline during continuous exposure of the cells to substance P. A second exposure to substance P after a 4–5 min drug-free wash produced a smaller response than the initial one (data not shown), demonstrating receptor desensitization. Because desensitization was particularly pronounced for substance P above $2 \times 10^{-8}$ M, we worked at $10^{-8}$ M substance P in the local anesthetic studies. The $Ca^{2+}$ response evoked by substance P and the binding of $^{125}$I-BHSP to membranes were significantly inhibited by the specific nonpeptide NK1 antagonist, CP-96345.[15]

Both the peak and the plateau calcium responses were significantly inhibited by 0.5 mM bupivacaine and virtually abolished by greater concentrations (fig. 6). The concentration-dependences of these inhibitions were indistinguishable, with $K_i$ values of approximately 0.5 mM bupivacaine (fig. 6), assuming a model of noncompetitive inhibition of substance P by bupivacaine (refer to fig. 6 legend). Furthermore, both $Ca^{2+}$ responses and agonist binding are inhibited by similar concentrations of local anesthetic (fig. 1).

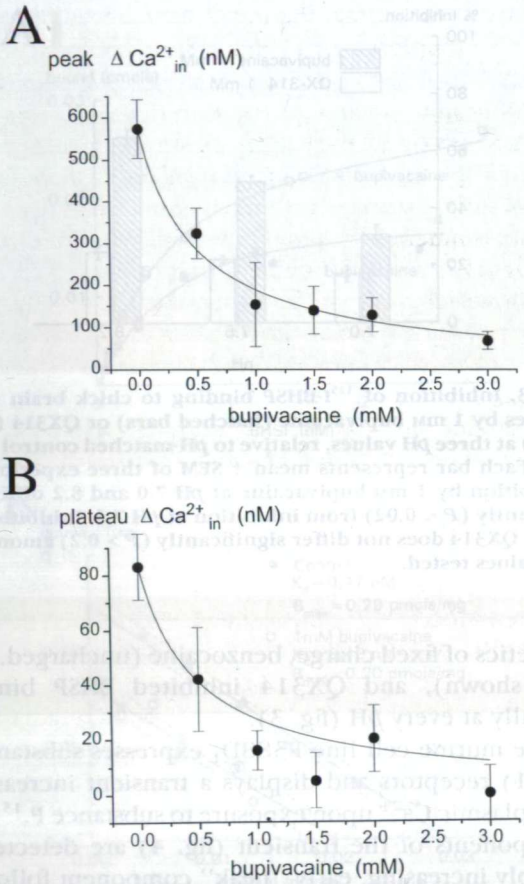


**Fig. 6. Inhibition of (*A*) peak $[Ca^{2+}]_{in}$ and (*B*) plateau $[Ca^{2+}]_{in}$ in response to $10^{-8}$ M substance P, *versus* bupivacaine. Bupivacaine at the concentration noted on the x-axis was present for 5 min before as well as during application of substance P. Points are mean ± SEM from three to five experiments. The solid line is a curve of the equation:**

$$[\Delta Ca^{2+}] = \frac{K_i}{K_i + [BUP]}[\Delta Ca^{2+}]_{MAX},$$

**where $[Ca^{2+}]_{MAX}$ is the response when bupivacaine is absent. A $K_i$ of 0.5 mM bupivacaine was assumed for both *A* and *B*.**

## Discussion

In the current studies, we showed that BHSP binding to NK1 receptors in fragmented brain membranes is inhibited by local anesthetics. For bupivacaine, the most potent of the local anesthetics tested, an $EC_{50}$ of about 1 mM was determined. A similar potency of about 0.5 mM was observed for bupivacaine's inhibitory action on substance P-induced increases in intracellular $Ca^{2+}$ in a cell line (murine P388D$_1$[15]) expressing substance P receptors. As in many secretory cells stimulated by transmitters or hormones, two phases of the $Ca^{2+}$ increase are evident. By analogy with other cells, the early transient probably corresponds to release from intracellular reservoirs of $Ca^{2+}$ and the later plateau phase to the entry of extracellular $Ca^{2+}$ through the plasmalemma.[17] Although bupivacaine could inhibit either of these $Ca^{2+}$ delivery pathways directly (*vide infra*), the fact that both are inhibited at the same concentrations as that for inhibition of BHSP binding required only that the local anesthetic is acting at the NK1 receptor.

The structure of substance P is identical in all avian and mammalian species examined,[7,8] and a single type of substance P receptor (NK1) has been cloned from each of the species thus far reported.[8] The extraordinary homology between substance P receptors across species (in localization,[9,10] general pharmacology,[7,13] and amino acid sequence[8]) makes it likely that similar effects of local anesthetics would be observed on substance P receptors in human dorsal horn.

Local anesthetics are known to affect many diverse membrane activities, including different types of ion channels and catalytic enzymes (see ref. 6 for review). In addition to a primary role in impulse blockade through the inhibition of $Na^+$ channels, local anesthetics inhibit voltage-gated $Ca^{2+}$ and $K^+$ channels, ATP-sensitive $K^+$ channels, and various ligand-gated channels. However, the potency rank order for these actions



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almost always follows the hydrophobicity ranking: tetracaine > bupivacaine > lidocaine > procaine > benzocaine.[18]

Inhibition of $Na^+$ channels is greater for the protonated species of local anesthetics in the cytoplasm,[19,20] whereas the neutral form is of limited but measurable potency.[21–23] Intracellular quaternary QX314, for example, is much more potent than its tertiary amine homolog lidocaine.[24,25] For the inhibition of BHSP binding, however, we observe the opposite behavior, with inhibition by bupivacaine ($pK_a = 8.2$) greater at more alkaline *p*H. In addition, QX314 and lidocaine are equipotent in antagonizing BHSP binding (table 1). Still, a finite activity for the protonated species is suggested by the *p*H-dependence of inhibition (fig. 3). This hypothesis is consistent with the *p*H-independent inhibition produced by the constantly charged cationic local anesthetic QX314 and the uncharged local anesthetic benzocaine.

Stereoselectivity for local anesthetic inhibition varies among the voltage-gated channels. Inhibition of $Na^+$ channels is greater with the R(+) than S(−) enantiomers.[26] The opposite stereoselectivity is seen for N-type $Ca^{2+}$ channels, and no stereoselectivity occurs for inhibition of delayed rectifier or transient outward $K^+$ channels.[27] BHSP binding also was inhibited without stereoselectivity.

Among the other membrane targets affected by local anesthetics, members of the superfamily of proteins with seven putative transmembrane domains that are coupled to G proteins are most germane. At first view, the results are complex and apparently contradictory. Both β-adrenoreceptors and muscarinic cholinergic receptors are inhibited by local anesthetics. For the adrenoreceptors, assayed in frog erythrocytes,[28] both agonist and antagonist binding and subsequent stimulation of adenylate cyclase were depressed by local anesthetics. The rank order of potency for inhibition of both agonist binding and cyclase activity was dibucaine > tetracaine > bupivacaine > lidocaine, which differs from the potency order for inhibition of substance P binding reported here (table 1). Another difference is in the mode of binding inhibition, which is not competitive for substance P binding (fig. 2) but is competitive for both agonist and antagonist binding to β-adrenoreceptors.[28] Results from a study of nonhydrolyzable guanine nucleotide analogs stimulating cyclase activity suggest an indirect, modulatory (allosteric) rather than a direct action by local anesthetics. Specifically, preincubation of membrane-bound receptors with isoproterenol and Gpp(NH)p led to a stimulation of adenylate cyclase activity that could be inhibited by tetracaine if it was present in the preincubation period but not if it was added after cyclase activation.[28] Other experiments showed that an inhibition of cyclase by tetracaine in the preincubation mixture depended on the concentration of guanine nucleotide and that the guanine nucleotide-induced dissociation of agonist was accelerated by tetracaine, in membrane-associated as well as detergent-solubilized receptors. Together, these results indicate an action of local anesthetics on the G-protein-binding/activating site of the adrenergic receptor molecule.[28]

In muscarinic cholinergic receptors, the inhibition of antagonist binding by local anesthetic also was competitive at low tetracaine concentrations; the calculated $K_i$ was ~2 μM.[29] At higher concentrations, purely competitive inhibition did not occur. The rank potency order for this competitive inhibition was tetracaine > procaine > bupivacaine > lidocaine, quite unlike that for inhibition of BHSP binding. Stimulation of cGMP formation by carbachol-activated muscarinic receptors in neuroblastoma cells also was inhibited by local anesthetics.[30] The inhibition was characterized as competitive with agonist and had the potency ranking tetracaine = procaine > lidocaine ≫ benzocaine.

In contrast to the net inhibitory actions described above, the effects of local anesthetics on receptor-stimulated cyclase activities are varied. In addition to the inhibition of systems mentioned above, local anesthetics enhance glucagon- and fluoride-stimulated adenylate cyclase in rat liver plasmalemma.[31] At ~$10^{-3}$ M both dibucaine and mepivacaine (a homologue of bupivacaine) increase $F^-$-stimulated cyclase activity; inhibition occurs at higher local anesthetic concentrations. Stimulation requires intact membranes and correlates with an anesthetic-induced increase of membrane fluidity, as measured by electron spin resonance studies of a spin-labeled fatty acid. The authors suggest that the local anesthetics effect an inhibition in enzyme activity by modifying the "coupling interaction" between receptor and catalytic unit. The role of local anesthetics acting directly at the receptor *versus* effects on receptor-effector coupling mediated by changes in membrane properties must be separated before the complete local anesthetic pharmacology can be understood.

We observed an apparently noncompetitive inhibition of BHSP binding by local anesthetics, whereas others have reported competitive inhibitions with other



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agonists in other systems. This difference may provide a clue to the mechanism of local anesthetic actions on these proteins. In the NK1 (substance P) receptors we studied, agonist binding is strongly modified by guanine nucleotides.[13] Guanine nucleotide reduces the BHSP affinity so much that specific agonist binding in its presence cannot be detected. In muscarinic and adrenergic systems, agonist affinity is modulated less strongly by guanine nucleotides, such that some agonist binding still occurs in their presence. If local anesthetics cause a large decrease in NK1 receptor affinity for agonist, to a level below detection under experimental conditions, the result will appear as a reduction in the number of binding sites, *i.e.,* a noncompetitive effect (fig. 2). The weaker effects of local anesthetics on agonist affinity of $\beta$-adrenergic and muscarinic receptors would permit some agonist binding in the presence of the local anesthetic; these conditions describe competitive inhibition.

It is likely that the actions on NK1 receptors described here occur during clinical anesthesia. During spinal nerve blocks, concentrated local anesthetic solutions are injected directly into the cerebrospinal fluid and diffuse into the nerve roots and spinal cord, where NK1 tachykinin receptors are concentrated in the dorsal horn.[8] In studies of the distribution of radiolabeled lidocaine during intrathecal anesthesia in dogs, Cohen showed that, at 30 min after drug injection, a time when anesthesia is well established, the lidocaine concentration in cerebrospinal fluid was 3.3 mM, and in the gray matter of the dorsal horn, the lidocaine content was 2.2 nmol/mg wet tissue.[4] This value corresponds to the uptake into peripheral nerve equilibrated with 2 mM lidocaine.[32,33] It is noteworthy that a dorsal-ventral gradient of lidocaine or procaine was apparent after intrathecal injections in the dog.[4] Dorsal columns contained twice the local anesthetic of the ventral columns, and the same ratio was found for dorsal roots to ventral roots. If a similar pattern occurs with spinal anesthesia in humans, it may explain in part why sensory block exceeds motor block in many patients,[1] considering that more potential targets for local anesthetics are present in the dorsal horn and that the drug may be more concentrated there.

Bromage *et al.*,[5] investigating the penetration of lidocaine into spinal cord from epidural administration to dogs, found an identical value of 2.2 nmol/mg wet tissue for the maximum uptake in cross-sections of spinal cord assessed autoradiographically. Bupivacaine usually is used at a lower dose than lidocaine and, given its greater hydrophobicity,[18] would be at proportionately higher content in the spinal tissue, *i.e.,* at levels corresponding to 0.5–1.0 mM free bupivacaine. These clinical concentrations of local anesthetics have substantial effects on substance P binding and substance P-evoked $Ca^{2+}$ responses *in vitro* (*vide supra*).

The general structure of local anesthetics bears a certain similarity to several nonpeptide antagonists of substance P.[34] Both types of molecules contain hydrophobic, aromatic regions linked to amino groups that are, at least in part, protonated at physiologic conditions. As might be predicted from this structural homology, at high concentrations (100-fold higher than employed in the current studies), these substance P antagonists show reversible impulse-blocking activity, including a "use-dependent" inhibition like that of traditional local anesthetics.[22,34]

Although local anesthetic-mediated actions in peripheral nerve probably are largely due to "classic" actions on $Na^+$ channels, the functional pharmacologic targets participating in epidural and intrathecal anesthesia are almost certainly broader. With the realization that local anesthetics can affect tachykinin receptors important for the neurotransmission of nociceptive signals, we should move to develop drugs for these procedures that act effectively on both channels and receptors. Such agents would be both more selective for nociception and more potent as analgesics.

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