

## ***Bupivacaine Inhibits Cyclic-3',5'-Adenosine Monophosphate Production***

### ***A Possible Contributing Factor to Cardiovascular Toxicity***

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**Background:** It was hypothesized that local anesthetic inhibition of cyclic-3',5'-adenosine monophosphate (cAMP) production may contribute to cardiovascular toxicity. This study was undertaken to determine whether bupivacaine is a more potent inhibitor of cAMP production than are chemically related local anesthetics that are less prone to produce cardiovascular toxicity.

**Methods:** Volunteers provided venous blood from which lymphocytes were isolated. Production of cAMP was measured under basal conditions, and in response to stimulation with either forskolin or epinephrine. Inhibition of basal, epinephrine-stimulated, and forskolin-stimulated cAMP production by mepivacaine, ropivacaine, and bupivacaine was assessed.

**Results:** Both forskolin and epinephrine produced concentration-dependent increases in cAMP production; the 50% effective concentrations ( $EC_{50}$ s) for these drugs were  $3.6 \times 10^{-8}$  and  $4.6 \times 10^{-8}$  M, respectively. Maximal forskolin-stimulated cAMP production ( $6.6 \pm 0.8$  pg/ $10^6$  cells/ $10$  min at  $10^{-6}$  M) was greater than maximal epinephrine-stimulated cAMP production ( $4.2 \pm 0.6$  pg/ $10^6$  cells/ $10$  min at  $10^{-5}$  M). Bupivacaine ( $IC_{50} = 2.3 \times 10^{-6}$  M) more potently inhibited basal cAMP production than either ropivacaine ( $IC_{50} = 4 \times 10^{-6}$  M) or mepivacaine ( $IC_{50} = 3.2 \times 10^{-5}$  M). Similarly, bupivacaine ( $IC_{50} = 2.3 \times 10^{-6}$  M) was as potent as ropivacaine ( $IC_{50} = 1.7 \times 10^{-6}$  M) and more potent than mepivacaine ( $IC_{50} = 8.9 \times 10^{-6}$  M) at inhibiting epinephrine-stimulated cAMP production. Bupivacaine ( $IC_{50} = 5.3 \times 10^{-6}$  M) was only marginally more potent than ropivacaine ( $IC_{50} = 9.7 \times 10^{-6}$  M) or mepivacaine ( $IC_{50} = 6.8 \times 10^{-6}$  M) at inhibition of forskolin-stimulated cAMP production.

Comparison of epinephrine concentration-response curves in the presence and absence of bupivacaine (0.35, 3.5, and 35  $\mu$ M) demonstrated noncompetitive inhibition of cAMP production by the local anesthetic.

**Conclusions:** Inhibition of basal and epinephrine-stimulated cAMP production may contribute to local anesthetic cardiovascular toxicity. Inhibition of cAMP production may limit the success of resuscitative measures and drugs administered for bupivacaine cardiovascular toxicity. Increasing the resuscitation dose of epinephrine may be required to restore cardiac contractile function after bupivacaine intoxication. (Key words: Anesthetic, local: bupivacaine; mepivacaine; ropivacaine. Enzymes: adenylyl cyclase. Forskolin. Metabolism: cyclic AMP. Sympathetic nervous system, catecholamines: epinephrine. Toxicity.)

CARDIOVASCULAR toxicity induced by local anesthetics is an uncommon, but often catastrophic, complication of regional anesthesia.<sup>1</sup> Most reported episodes of severe local anesthetic-induced cardiovascular toxicity have occurred after accidental intravenous injection of bupivacaine during attempted epidural or brachial plexus anesthesia, techniques requiring relatively large volumes of local anesthetic. In contrast to other, less potent local anesthetics that depress cardiac contractility only at higher concentrations, bupivacaine depresses ventricular contractility *in vitro* at relatively low concentrations.<sup>2-5</sup> Resuscitation after bupivacaine cardiovascular toxicity has been reported to be more difficult than after other local anesthetics; this is, perhaps, related to the need for different drugs than those included in standard resuscitation protocols.<sup>6,7</sup>

We hypothesized that local anesthetic inhibition of a standard resuscitation drug may contribute to bupivacaine cardiovascular toxicity. In our hypothesis, bupivacaine, after initially producing toxicity (whether as a consequence of arrhythmias, reduced myocardial contractility, vasomotor paralysis, inhibition of medullary vasomotor centers, or inadequate airway support

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Received from the Department of Anesthesia, Section on Cardiothoracic Anesthesia, The Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina. Accepted for publication March 2, 1993. Supported in part by grants from the North Carolina Society of Anesthesiologists (RCB) and from the Foundation for Anesthesia Education and Research (RCP).

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after a generalized seizure), may exacerbate the cardiovascular depression by reducing the ability of epinephrine to support the circulation. We tested this hypothesis using the  $\beta$ -adrenergic receptor-guanine nucleotide binding (G) protein-adenylyl cyclase complex found within the membranes of human lymphocytes as our model for  $\beta$ -adrenergic responses in the human heart. We compared basal and epinephrine-stimulated cAMP production before and after bupivacaine, mepivacaine, and ropivacaine, local anesthetics with similar chemical structure to bupivacaine. Finally, in an attempt to localize the site of local anesthetic action, we also examined local anesthetic inhibition of forskolin-stimulated cAMP production. Forskolin, unlike  $\beta$ -adrenergic agonists, directly activates adenylyl cyclase without the need for binding to a cell-surface receptor or for transduction *via* G proteins.

### Materials and Methods

Twenty-five to 30 ml of venous blood was withdrawn aseptically from volunteers (46 women, 17 men), and immediately anticoagulated with heparin. The volunteers ranged in age from 21 to 45 years, and ranged in weight from 52 to 95 kg. No volunteer had a medical condition that, by itself or with treatment, was known to influence  $\beta$ -adrenergic receptor responses (*e.g.*, heart failure, hypertension, obstructive airway disease, or coronary artery disease).<sup>8</sup> More specifically, no subject was receiving  $\beta$ -adrenergic agonists or antagonists, or any form of bronchodilator. Blood from only one volunteer was used in any one lymphocyte preparation.

Lymphocytes were isolated using a modification of the method of Böyum.<sup>9</sup> Heparinized blood was applied to Ficol-Paque (Pharmacia LKB Biotechnology, Piscataway, NJ) and subjected to density-gradient centrifugation at 1,300 g for 35 min. The lymphocyte-rich fraction was collected from the Ficol-Paque, suspended in phosphate buffered saline (at pH = 6 and room temperature), and centrifuged at 1,300 g for 5 min. The phosphate buffered saline solution contained 140 mM NaCl, 2.7 mM KCl, 1.5 mM  $\text{KH}_2\text{PO}_4$ , and 6 mM  $\text{K}_2\text{HPO}_4$ . The supernatant was discarded; the pellet was resus-

ended in phosphate buffered saline. Cell counts were accomplished using a Model ZE cell counter set to recognize only mononuclear cells (Coulter Electronics, Hialeah, FL). The final suspension contained 2–4 million mononuclear cells (which we refer to as lymphocytes) per milliliter. Previous studies have shown that the mononuclear cells include: ~70% T-lymphocytes (positive for cluster designation 3 [CD3] antigen), ~10% B-lymphocytes (positive for CD19 antigen), and ~15% monocytes, macrophages, and polymorphonuclear cells (positive for CD14 antigen).<sup>10</sup>

Aliquots of the lymphocyte suspension were added to 50  $\mu\text{M}$  isobutylmethylxanthine (Sigma Chemical Company, St. Louis, MO) and the appropriate local anesthetic and/or agonist concentration. Isobutylmethylxanthine inhibited the various cyclic nucleotide phosphodiesterase forms found within lymphocytes. Thus, our assays measured the rate of cAMP production; enzymatic cAMP lysis was completely inhibited by isobutyl-methylxanthine.<sup>11</sup> After 10 min, the reaction was quenched using perchloric acid. Cyclic AMP was assessed by competitive protein binding using radioimmunoassay (Kit APH2-0033; Diagnostic Products Corporation, Los Angeles, CA) in an adaptation of the method of Tovey *et al.*<sup>13</sup> All daily assays were performed in duplicate; each data point represented the mean of these two determinations.

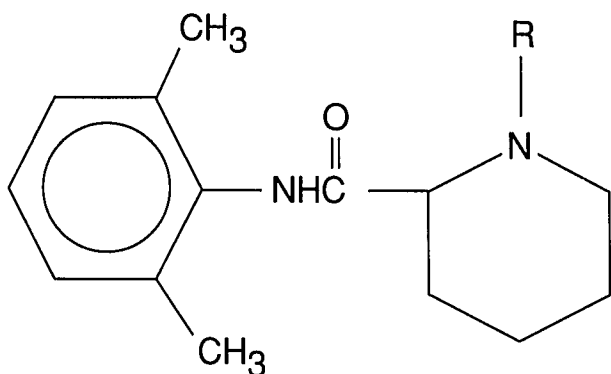
Inhibition of cAMP production by drugs was measured by comparing two identical samples (from the same blood donor), with the only difference being the presence in one sample of the inhibitor. All chemicals were reagent grade or better and were purchased from commercial sources. Racemic hydrochloride salts of bupivacaine and mepivacaine were provided by Dr. Bertil Takman, formerly of Astra Pharmaceuticals, Westboro, MA (fig. 1). Ropivacaine hydrochloride was tested in its clinical formulation (the S(-) isomer). Data were plotted and analyzed using Inplot (Graphpad Software, San Diego, CA). The  $\text{EC}_{50}$  and  $\text{IC}_{50}$  were calculated by nonlinear regression of individual data points (not the mean data) to a four-parameter, sigmoidal, logistic equation of the form:

$$Y = A + \frac{B - A}{1 + \left(\frac{10^C}{10^X}\right)^H}$$

where Y = the drug response; A = the bottom plateau; B = the top plateau; C = the logarithm of the midpoint (the 50% effective concentration =  $\text{EC}_{50}$ , or the 50% inhibitory concentration =  $\text{IC}_{50}$ ); X = the logarithm of

§ The total accumulation of cAMP (after exposure to agonists) is related to the duration of drug exposure; however, receptor desensitization and agonist inactivation is also time dependent.<sup>12</sup> In preliminary experiments (data not shown), a 10-min reaction time was judged to give reproducible cAMP accumulation and was used in all studies. Moreover, the 10-min reaction time limited the opportunity for lymphocyte desensitization and epinephrine metabolism.<sup>12</sup>

## 1-alkyl-2', 6'-pipecoloxylidide



| R               | -CH <sub>3</sub> | -C <sub>3</sub> H <sub>7</sub> | -C <sub>4</sub> H <sub>9</sub> |
|-----------------|------------------|--------------------------------|--------------------------------|
| Name            | Mepivacaine      | Ropivacaine                    | Bupivacaine                    |
| MW              | 246              | 274                            | 288                            |
| pK <sub>a</sub> | 7.9              | 8.2                            | 8.2                            |
| P <sup>+</sup>  | .1               | .5                             | 1.5                            |
| P <sup>0</sup>  | 90               | 780                            | 2570                           |

Fig. 1. Chemical structures, molecular weights, pK<sub>a</sub>, and octanol:buffer partition coefficients (P<sup>+</sup> = charged [or protonated] local anesthetic; P<sup>0</sup> = uncharged [or neutral] local anesthetic) for mepivacaine, ropivacaine, and bupivacaine. Note that the three local anesthetics represent different alkyl substitutions on the pipecoloxylidide parent compound, and that their lipid solubility (see partition coefficients for both the neutral and protonated local anesthetic species) increases with increasing molecular weight. Mepivacaine and bupivacaine were studied as racemic mixtures; ropivacaine was studied (in its clinical formulation) as the S(-) isomer. (Data adapted from reference 38.)

drug concentration; and H = the Hill coefficient. When IC<sub>50</sub> values for local anesthetics were calculated, A was fixed at 0%, B was fixed at 100%, and the Hill coefficient was fixed at -1. When EC<sub>50</sub> values for epinephrine and forskolin stimulation of cAMP production were determined, A and B were allowed to "float" and the Hill coefficient was fixed at +1. Data are presented as the means and 95% confidence limits or as means ± SEM. Our protocol was reviewed and approved by the Clinical Research Practices Committee for the North Carolina Baptist Hospital and Bowman Gray School of Medicine of Wake Forest University. Each blood donor gave his or her written informed consent to participate in the study.

## Results

*Epinephrine and Forskolin Stimulation of cAMP Production*

Both epinephrine and forskolin, in the absence of local anesthetics, produced concentration-dependent increases in cAMP production in lymphocytes (fig. 2). Forskolin was more effective than epinephrine at stimulating cAMP production. Forskolin's EC<sub>50</sub> ( $3.6 \times 10^{-8}$  M, 95% confidence limits  $1.4 \times 10^{-8}$  M to  $9.6 \times 10^{-8}$  M) was slightly less than that of epinephrine ( $4.6 \times 10^{-8}$  M, 95% confidence limits  $1.0 \times 10^{-8}$  M to  $2.0 \times 10^{-7}$  M); moreover, maximal forskolin-stimulated cAMP production ( $6.6 \pm 0.8$  pg/10<sup>6</sup> cells/10 min at  $10^{-6}$  M) was considerably greater than the maximal response to epinephrine ( $4.2 \pm 0.6$  pg/10<sup>6</sup> cells/10 min at  $10^{-5}$  M).

*Concentration-Dependent Local Anesthetic Inhibition of cAMP Production*

The three local anesthetics inhibited basal cAMP production in lymphocytes (fig. 3). Basal cAMP production was  $0.59 \pm 0.06$  pg/10<sup>6</sup> cells/10 min (before bupi-

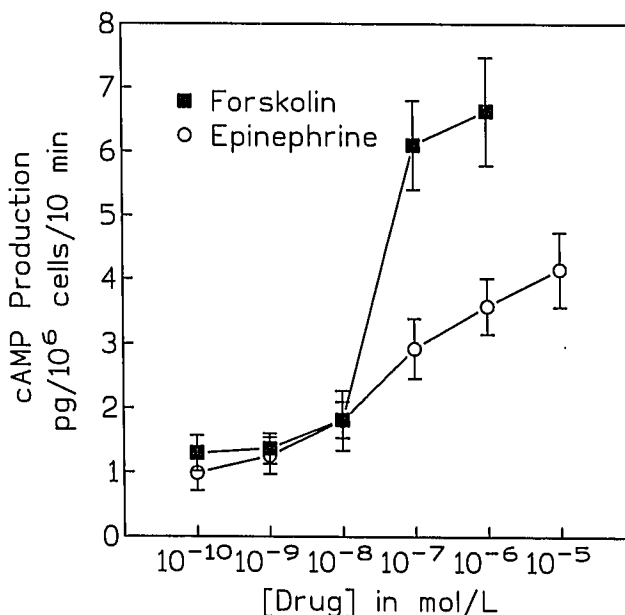
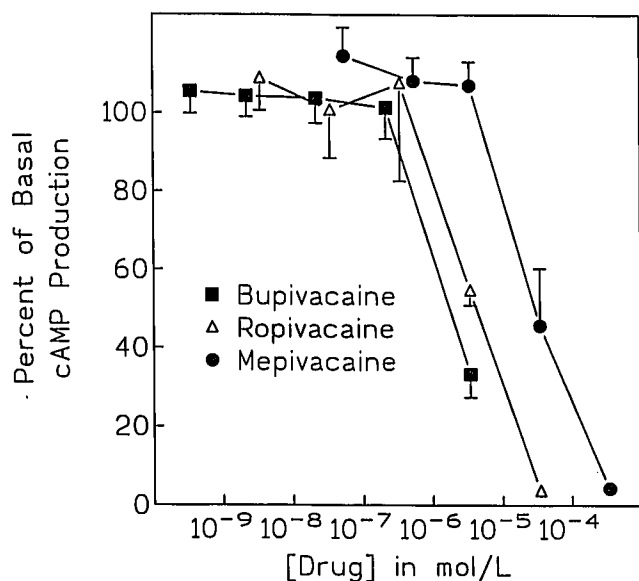


Fig. 2. Stimulation of cAMP production by either forskolin or epinephrine. Data are presented as means ± SEM. For epinephrine, data from eight experiments are reported; for forskolin, data from ten experiments are reported. The EC<sub>50</sub> of epinephrine =  $4.6 \times 10^{-8}$  M, and the EC<sub>50</sub> of forskolin =  $3.6 \times 10^{-8}$  M. Forskolin produced a greater maximal cAMP response ( $6.6 \pm 0.8$  pg/10<sup>6</sup> cells/10 min) than epinephrine ( $4.2 \pm 0.6$  pg/10<sup>6</sup> cells/10 min) and had a lower EC<sub>50</sub>.

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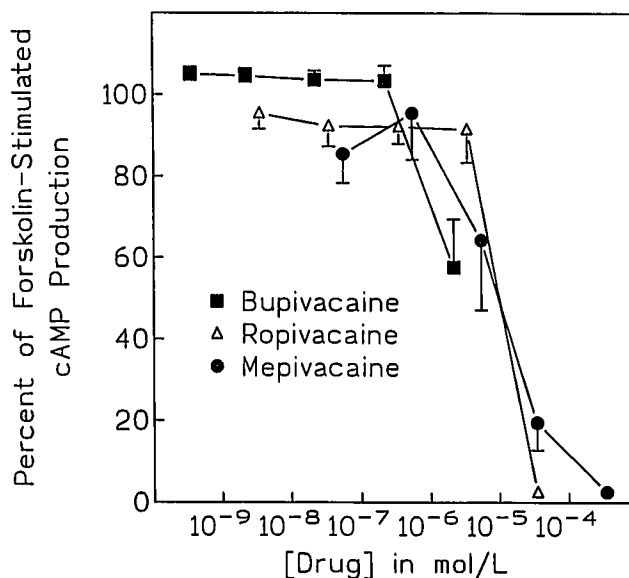
**Fig. 3.** Inhibition of basal cAMP production by mepivacaine, ropivacaine, or bupivacaine. Cyclic cAMP production in the presence of local anesthetics is given as the percent of the cAMP production observed in the absence of the local anesthetic. Data are presented as means  $\pm$  SEM. For mepivacaine, data from 6 experiments are reported; for ropivacaine, data from 6 experiments are reported; and for bupivacaine, data from 20 experiments are reported. To prevent overlap, some bupivacaine and mepivacaine data points have been slightly offset. The ropivacaine symbol precisely identifies the drug concentrations we used. Bupivacaine was slightly more potent than ropivacaine; both bupivacaine and ropivacaine were considerably more potent than mepivacaine. The  $IC_{50}$  of mepivacaine =  $3.2 \times 10^{-5}$  M, the  $IC_{50}$  of ropivacaine =  $4 \times 10^{-6}$  M, and the  $IC_{50}$  of bupivacaine =  $2.3 \times 10^{-6}$  M.

vacaine),  $0.57 \pm 0.09$  pg/ $10^6$  cells/10 min (before ropivacaine), and  $0.47 \pm 0.14$  pg/ $10^6$  cells/10 min (before mepivacaine). The calculated  $IC_{50}$  values were: bupivacaine  $2.4 \times 10^{-6}$  M (95% confidence limits  $1.6 \times 10^{-6}$  M to  $4.0 \times 10^{-6}$  M), ropivacaine  $4.5 \times 10^{-6}$  M (95% confidence limits  $1.6 \times 10^{-6}$  M to  $1.3 \times 10^{-5}$  M), and mepivacaine  $3.2 \times 10^{-5}$  M (95% confidence limits  $1.7 \times 10^{-5}$  M to  $6.0 \times 10^{-5}$  M).

The three local anesthetics inhibited forskolin-stimulated cAMP production (fig. 4). Forskolin stimulation at  $5 \times 10^{-8}$  M (approximately the  $EC_{50}$ ) yielded cAMP production of  $3.9 \pm 1.0$  pg/ $10^6$  cells/10 min (before bupivacaine),  $3.0 \pm 1.0$  pg/ $10^6$  cells/10 min (before ropivacaine), and  $2.2 \pm 0.6$  pg/ $10^6$  cells/10 min (before mepivacaine). The variable responses to forskolin stimulation (alone) are the result of varying basal cAMP production in a varying donor pool. The calculated  $IC_{50}$  values were: bupivacaine  $5.3 \times 10^{-6}$  M (95% confidence limits  $3.2 \times 10^{-6}$  M to  $8.7 \times 10^{-6}$  M), ropiva-

caine  $9.7 \times 10^{-6}$  M (95% confidence limits  $5.2 \times 10^{-6}$  M to  $1.8 \times 10^{-5}$  M), and mepivacaine  $6.8 \times 10^{-6}$  M (95% confidence levels  $3.2 \times 10^{-6}$  M to  $1.5 \times 10^{-5}$  M).

The three local anesthetics inhibited epinephrine-stimulated cAMP production (fig. 5). Epinephrine stimulation at  $10^{-7}$  M (a concentration approximating the calculated  $EC_{50}$ ) yielded cAMP production of  $2.4 \pm 0.4$  pg/ $10^6$  cells/10 min (before bupivacaine),  $1.4 \pm 0.3$  pg/ $10^6$  cells/10 min (before ropivacaine), and  $2.2 \pm 0.7$  pg/ $10^6$  cells/10 min (before mepivacaine). Again, the variable responses to epinephrine stimulation were expected, given the varying basal cAMP production in the varying donor pool. The calculated  $IC_{50}$  values were: bupivacaine  $2.3 \times 10^{-6}$  M (95% confidence limits  $1.7 \times 10^{-6}$  M to  $3.2 \times 10^{-6}$  M), ropivacaine  $1.7 \times 10^{-6}$  M (95% confidence limits  $1.0 \times 10^{-6}$  M to  $2.7 \times 10^{-6}$  M), and mepivacaine  $8.9 \times 10^{-6}$  M (95% confidence limits  $6.0 \times 10^{-6}$  M to  $1.3 \times 10^{-5}$  M).



**Fig. 4.** Inhibition of forskolin-stimulated cAMP production by mepivacaine, ropivacaine, or bupivacaine. The cAMP production in the presence of local anesthetics is given as the percent of the cAMP production observed with application of forskolin ( $5 \times 10^{-8}$  M) in the absence of the local anesthetic. Data are presented as means  $\pm$  SEM. For mepivacaine, data from four experiments are reported; for ropivacaine, data from five experiments are reported; for bupivacaine, data from five experiments are reported. To prevent overlap, some bupivacaine and mepivacaine data points have been slightly offset. The ropivacaine symbol precisely identifies the drug concentrations we used. The  $IC_{50}$  of mepivacaine =  $6.8 \times 10^{-6}$  M, the  $IC_{50}$  of ropivacaine =  $9.7 \times 10^{-6}$  M, and the  $IC_{50}$  of bupivacaine =  $5.3 \times 10^{-6}$  M.

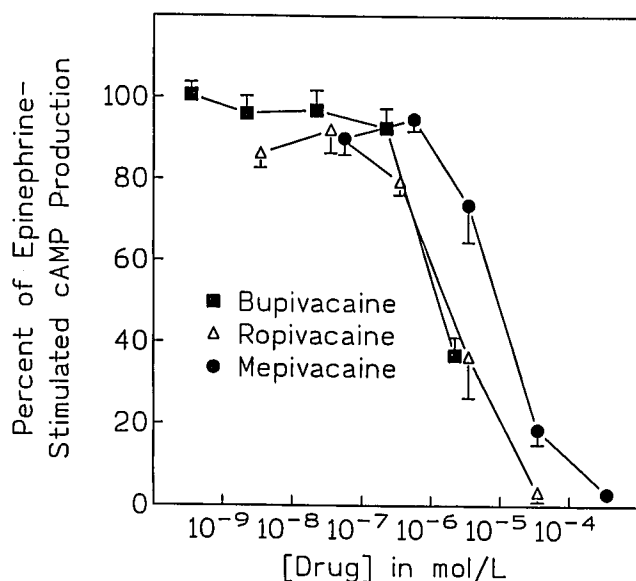


Fig. 5. Inhibition of epinephrine-stimulated cAMP production by mepivacaine, ropivacaine, or bupivacaine. The cAMP production in the presence of local anesthetics is given as the percent of the cAMP production observed with application of epinephrine ( $10^{-7}$  M) in the absence of the local anesthetic. Data are presented as means  $\pm$  SEM. For mepivacaine, data from 4 experiments are reported; for ropivacaine, data from 5 experiments are reported; for bupivacaine, data from 16 experiments are reported. To prevent overlap, some bupivacaine and mepivacaine data points have been slightly offset. The ropivacaine symbol precisely identifies the drug concentrations we used. The  $IC_{50}$  of mepivacaine =  $8.9 \times 10^{-6}$  M, the  $IC_{50}$  of ropivacaine =  $1.7 \times 10^{-6}$  M, and the  $IC_{50}$  of bupivacaine =  $2.3 \times 10^{-6}$  M.

#### Effect of Bupivacaine on Epinephrine Concentration-Response Curve

Because epinephrine is often administered in cases of bupivacaine intoxication, the effect of bupivacaine on responses to increasing doses of epinephrine was measured to determine whether the antagonism was competitive (fig. 6). Bupivacaine demonstrated noncompetitive inhibition of epinephrine-stimulated cAMP production. Bupivacaine at 3.5 and 35  $\mu$ M reduced the maximal, epinephrine ( $10^{-5}$  M)-stimulated cAMP production to less than that produced by subtherapeutic concentrations of epinephrine ( $10^{-10}$  M) in the absence of bupivacaine. These data do not identify the locus of the antagonism between local anesthetics and epinephrine.

#### Discussion

Our data demonstrate that the three 1-alkyl-2',6'-piperidoloxylidide local anesthetics, mepivacaine, ropi-

vacaine, and bupivacaine, inhibit basal and epinephrine-stimulated cAMP production with a rank order of potency similar to that reported for their production of cardiovascular toxicity.<sup>1-5,14,15</sup> Thus, bupivacaine, which is prone to produce cardiovascular toxicity, more potently inhibited cyclic adenosine monophosphate (cAMP) production than mepivacaine, which is less prone to produce cardiovascular toxicity (fig. 1). Ropivacaine was nearly as potent as bupivacaine in inhibiting basal and epinephrine-stimulated cAMP production. We believe that local anesthetic inhibition of cAMP production contributes to difficult resuscitation after cardiovascular toxicity. Indeed, in the presence of toxic bupivacaine concentrations, even  $10^{-5}$  M of epinephrine could not increase cAMP production to levels achieved by subtherapeutic concentrations of epinephrine in the absence of bupivacaine.

Our data and our hypothesis regarding the importance of cAMP production in local anesthetic cardiovascular toxicity are consistent with clinical reports and animal studies indicating that mepivacaine and (perhaps) ropivacaine are relatively less likely to produce cardiovascular depression than bupivacaine,<sup>1-5,14,15</sup> and with animal studies demonstrating the efficacy of isoproterenol and amrinone at resuscitation from bupivacaine toxicity.<sup>16,17</sup> Although direct comparisons between drug concentrations used in *in vitro* studies and those obtained *in vivo* are often not feasible because of dif-

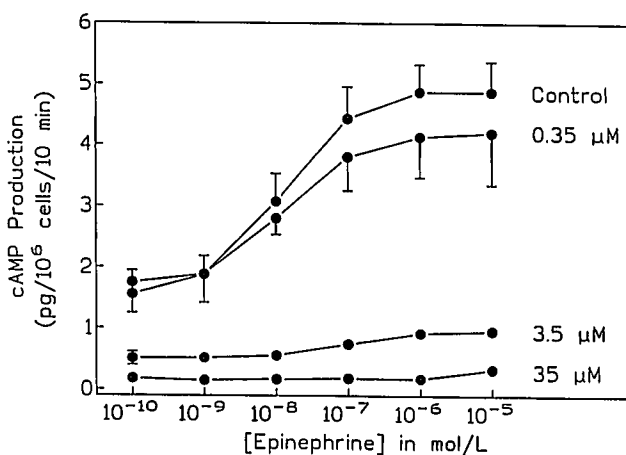


Fig. 6. Noncompetitive inhibition of epinephrine-stimulated cAMP production by bupivacaine. Experiments ( $n = 4$ ) were included in the absence of bupivacaine (control), and in the presence of 0.35, 3.5, and 35  $\mu$ M bupivacaine. Bupivacaine concentrations of 3.5 and 35  $\mu$ M reduced cAMP production (even in the presence of  $10^{-5}$  M epinephrine) to less than that produced by subtherapeutic concentrations of epinephrine ( $10^{-10}$  M) in the absence of the local anesthetic. Data are presented as means  $\pm$  SEM.

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ferences in drug access to binding sites, it should be noted that drug concentrations previously reported to reduce cardiac contractility (*e.g.*, 40  $\mu\text{M}$  mepivacaine or 5  $\mu\text{M}$  bupivacaine) are in the same range of concentrations at which we measured inhibition of cAMP production.<sup>1,2</sup>

There was less separation between bupivacaine and mepivacaine  $\text{IC}_{50}$  values for inhibition of forskolin-stimulated cAMP production than for inhibition of basal and epinephrine-stimulated cAMP production. Therefore, local anesthetic inhibition with adenylyl cyclase *per se* seems less likely to be the mechanism for difficult resuscitation after local anesthetic intoxication than local anesthetic inhibition of a more proximal step in the cAMP synthesis pathway. Studies are in progress to determine whether inhibition may occur through inhibition of binding to the  $\beta$ -adrenergic receptor.

We used epinephrine and forskolin to stimulate cAMP production because they represent two paradigms. Epinephrine, the archetypical  $\beta$ -adrenergic agonist, is the agent of choice for resuscitation after cardiac arrest. Local anesthetic inhibition of cAMP production could result from antagonism of receptor binding, antagonism of transduction, or inhibition of adenylyl cyclase. Alternatively, local anesthetics could stimulate the  $G_i$  inhibitory pathway. Forskolin stimulates cAMP production by directly stimulating adenylyl cyclase.<sup>18</sup> We used concentrations of these agonists that approximate their  $\text{EC}_{50}$  values, so that any local anesthetic-induced inhibition (or accentuation) of cAMP production would be readily apparent. The three local anesthetics inhibited forskolin-stimulated cAMP production with roughly equivalent potency, indicating the possibility that local anesthetics may directly interfere with the activity of adenylyl cyclase.

The lack of potency difference among the anesthetics makes us skeptical that this mechanism is important in clinical toxicity. We recognize that local anesthetics may have multiple sites of action on this system. The data we report do not permit us to identify with precision the site of local anesthetic inhibition of cAMP production. However, studies are in progress to better define local anesthetic interaction with this system.

### *Local Anesthetic Interactions with cAMP Metabolism*

Stimulation of  $\beta$ -adrenergic receptors leads to increased cAMP production. Elevated intracellular concentrations of cAMP mediate the positive inotropic and chronotropic actions of  $\beta$ -adrenergic receptor ago-

nists.<sup>19-21</sup> Interaction between local anesthetics and the  $\beta$ -adrenergic receptor-coupled adenylyl cyclase was examined in frog erythrocytes.<sup>22</sup> Tetracaine (a local anesthetic of comparable potency to bupivacaine), < 1 mM, increased isoproterenol stimulation of adenylyl cyclase, whereas > 1 mM tetracaine inhibited isoproterenol-stimulated cAMP production. In a few experiments, bupivacaine, tetracaine, and other local anesthetics competitively inhibited specific binding of  $\beta$ -adrenergic receptor agonists, identifying the receptor as a possible site for interaction between cAMP production and local anesthetics. In another study, mepivacaine increased fluoride-stimulated adenylyl cyclase, but had no effect on glucagon-stimulated adenylyl cyclase in rat hepatocytes.<sup>23</sup> These studies could be interpreted as identifying a local anesthetic binding site "proximal" to the catalytic subunit of adenylyl cyclase (*e.g.*, at or near the receptor).

Local anesthetics interfere with binding of the selective  $\beta$ -adrenergic antagonist <sup>3</sup>H-dihydroalprenolol (DHA) to smooth muscle membranes. Local anesthetics displace DHA from *nonspecific* sites with the same rank order of potency as for impulse blockade in nerves.<sup>24</sup> The importance of this nonstereospecific, yet displaceable, binding is unknown. In summary, local anesthetic interaction with adenylyl cyclase may occur at a site near the catalytic subunit. However, previous studies have not systematically examined adenylyl cyclase interactions between bupivacaine (and related local anesthetics) and agonist-stimulated cAMP production.

### *Assumptions and Limitations of the Study*

We assume that local anesthetic actions on human lymphocyte membranes model the responses of the human cardiac  $\beta$ -adrenergic receptor-adenylyl cyclase system. Lymphocytes contain a nearly pure population of  $\beta_2$ -adrenergic receptors, but the human heart contains a variable mixture of  $\beta_1$  and  $\beta_2$  receptors, the exact proportion of which depends on the region of the heart studied and on whether chronic disease (thyroid disease, cardiac ischemia, or chronic congestive heart failure) is present.<sup>25</sup> We could have used animal cardiac tissue as an alternative model of human cardiac responses; however, the rat ventricle (often used as a source of cardiac tissue) contains a nearly pure population of  $\beta_1$  receptors.<sup>26</sup> But there is no evidence that transduction mechanisms in  $\beta_1$  receptors are in any way different from those of  $\beta_2$  receptors.<sup>8,19,20,25,26</sup> Moreover, in the human heart, adenylyl cyclase is preferentially activated by  $\beta_2$  receptors, despite  $\beta_1$  receptors

being more numerous.<sup>27</sup> We believe that the lymphocyte assay represents a reasonable compromise and may be the best available model for *human*  $\beta_2$  adrenergic responses. We do plan to confirm our results in a tissue rich in  $\beta_1$  receptors.

We assume that the inhibition of cAMP production observed *in vitro* would also be observed *in vivo*. We chose local anesthetic concentrations that bracket those that have been measured in studies of cardiovascular toxicity. The molar potency ratio between bupivacaine and mepivacaine (for impulse blockade) is on the order of 3:1.<sup>1,28</sup> In clinical regional anesthesia, a threefold higher concentration of mepivacaine than bupivacaine is often used. The potency difference between bupivacaine and mepivacaine at inhibition of cAMP production was of at least this magnitude. Ropivacaine, with a potency at impulse blockade approximately equal to that of bupivacaine, approached bupivacaine in potency at inhibition of cAMP production.<sup>29</sup> Thus, it is unclear whether ropivacaine may be less prone than bupivacaine to inhibit the action of epinephrine after an accidental intravenous injection.

Our study does not define kinetic differences between the local anesthetics in their inhibition of cAMP formation. Thus, we recognize that we have not tested whether cells (or patients) pretreated with local anesthetics may show more inhibition of agonist-stimulated cAMP formation than we observed when local anesthetic and agonist were applied concurrently. Such differences between local anesthetics, if present, could have clinical importance.

Previous studies showing that inclusion of epinephrine with bupivacaine reduces the bupivacaine dose that produces cardiac arrest and death in animals,<sup>30</sup> and that pretreatment with propranolol increases the lethal bupivacaine dose, could be used as an argument against our hypothesis.<sup>31-33</sup> However, these LD<sub>50</sub> studies do not differentiate between local anesthetic-induced depression of contractile function (*e.g.*, by inhibition of cAMP production) and arrhythmogenesis as etiologies of bupivacaine cardiovascular toxicity. Moreover, we do not believe that pretreatment studies are necessarily relevant to a discussion of resuscitation after bupivacaine overdosage. Indeed, relative inhibition of  $\beta$ -adrenergic function (by bupivacaine) with preservation of  $\alpha$ -adrenergic function could explain why studies support the use of isoproterenol and amrinone to overcome the electrophysiologic and contractile effects of bupivacaine.<sup>16,17</sup> We believe that determining why standard resuscitative agents are relatively inef-

fective after bupivacaine toxicity may be as important as determining the etiology of bupivacaine toxicity *per se*.

Finally, we recognize that cardiovascular toxicity from local anesthetics may result from electrophysiologic, as well as contractile, actions of the agents. It seems reasonable (but remains unproven) that arrhythmias after local anesthetic overdosage result from local anesthetic binding to cardiac Na<sup>+</sup> channels. At the cellular level,  $\beta$ -adrenergic receptor agonists and other agents that increase intracellular cAMP lead to increased Na<sup>+</sup> channel inactivation, reducing the Na<sup>+</sup> flux.<sup>34-37</sup> There are, as yet, no data available showing whether  $\beta$ -adrenergic receptor agonists will attenuate or potentiate Na<sup>+</sup> channel inhibition by local anesthetics.

Bupivacaine inhibits basal and agonist-stimulated cAMP production more potently than mepivacaine. Greater potency of bupivacaine is consistent with previous studies of local anesthetic interaction with enzymes, which typically demonstrate a rank order of potency that follows their lipid solubility.<sup>25,28,38</sup> Furthermore, inhibition of epinephrine action represents a means by which resuscitation after bupivacaine intoxication may be rendered more difficult. Our data would tend to support the use of much-larger-than-normal resuscitation doses of epinephrine (or agents working through other pathways, such as amrinone<sup>17</sup>) for increasing cardiac contractile function after bupivacaine intoxication.

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