Local Anesthetics and the Role of Sodium in the Force Development by Canine Ventricular Muscle and Purkinje Fibers

MARIO VASSALLE AND MOHIT BHATTACHARYYA

SUMMARY We studied the effects of the local anesthetics, procaine and benzocaine, on the action potential and force of canine ventricular muscle and Purkinje fibers. In ventricular muscle, procaine often shifted the plateau to a more positive value and increased the force of contraction, whereas benzocaine shortened the action potential and decreased contractile force. In Purkinje fibers, both local anesthetics reduced contractile force and decreased the duration of the action potential markedly. In ventricular muscle fibers norepinephrine potentiated the positive inotropic effect of procaine and changed the effects of benzocaine from a negative to a positive inotropic effect. The potentiating effect of procaine on contraction was eliminated by propranolol. In the presence of norepinephrine (and the absence of propranolol), in Purkinje fibers both anesthetics decreased force but less than in the absence of norepinephrine. In the presence of tetrodotoxin (TTX) and norepinephrine, procaine and benzocaine increased contractile force of muscle fibers. Under the same conditions in Purkinje fibers, procaine increased contractile force and benzocaine decreased it (but less than in the absence of TTX). Administration of veratridine increased contractile force in both ventricular muscle and Purkinje fibers: under these conditions, both local anesthetics decreased contractile force in both tissues. We conclude that: (1) the positive inotropic effects of procaine and benzocaine are mediated through an adrenergic mechanism; (2) the negative inotropic effect is at least in part mediated through a reduction of sodium influx; and (3) the negative inotropic effect is more pronounced in Purkinje fibers because of a larger steady state sodium influx during the plateau.

LOCAL anesthetics block the fast sodium channel in Purkinje fibers and markedly shorten the action potential (Weidmann, 1955a). Recent experiments from our laboratory have shown that procaine and benzocaine also markedly decrease the force of contraction in this same tissue (Bhattacharyya and Vassalle, 1979). One possible explanation for these findings is that local anesthetics decrease both the duration of the action potential and the force of contraction by decreasing the sodium influx. The decrease in duration of the action potential would be expected because Purkinje fibers have a steady state tetrodotoxin-sensitive sodium current during the plateau (Attwell et al., 1979). The decrease in contractile force would be expected if a decrease in sodium influx results in a decreased intracellular concentration of sodium and, therefore (Blaustein, 1974; Langer, 1977), of calcium.

If local anesthetics act on contractile force by decreasing sodium influx, the effect on ventricular muscle fibers should be less pronounced than in Purkinje fibers, since the sodium influx appears to be much less in the former than in the latter tissue (Coraboeuf et al., 1979). Furthermore, if the negative inotropic effect of local anesthetics involves a decrease in sodium influx, this effect should vary if the sodium influx is varied and should become smaller when force of contraction is enhanced by enhancing calcium influx.

We approached the problem of the role of sodium on force development by investigating the action of procaine and benzocaine in ventricular muscle and Purkinje fibers from the same hearts under the same experimental conditions.

Methods

Mongrel dogs of either sex (12–20 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv), and the heart was excised through an intercostal incision. Strands of Purkinje fibers and small trabeculae carneae or papillary muscles (diameters less than 1 mm and lengths of 4 to 8 mm) were removed from both ventricles and were superfused with oxygenated (97% O2, 3% CO2) Tyrode’s solution maintained at 37°C. The Tyrode solution had the following composition (in mM): NaCl, 136.9; KCl, 2.7; NaHCO3, 11.9; Na2HPO4, 0.45; MgCl2, 0.5; CaCl2, 2.7; dextrose, 5.5.

A Grass S4 stimulator connected to a Grass SIU 4678 stimulus isolation unit was used to drive the fibers at a constant rate of 60/min. The output of the stimulus isolation unit was connected to two...
The Effects of Procaine and Benzocaine on the Mechanical and Electrical Events in Myocardial and Purkinje Fibers

In ventricular muscle, procaine most often increased the force of contraction, whereas benzocaine usually decreased it. In Purkinje fibers, procaine and benzocaine decreased or abolished force.

The action potential and twitch in the absence and presence of the local anesthetics are illustrated in Figure 1. Procaine shifted the plateau to less negative values and shortened the action potential slightly, whereas the amplitude of the twitch increased (Fig. 1A). Benzocaine did not much affect the plateau but shortened the action potential and decreased the force of contraction (Fig. 1B). In Purkinje fibers, both procaine (C) and benzocaine (D) markedly shortened the action potential and decreased the force of contraction, as expected.

In ventricular muscle fibers, procaine increased the force in 12 experiments (+51.3 ± 13.3%) and decreased it (−12.2 ± 3.4%) in five experiments. Benzocaine decreased the force (−39.7 ± 5.7%) in 14 experiments and increased it (+4 ± 2.1%) in two experiments. In Purkinje fibers, these local anesthetics consistently decreased the force. The average values for all experiments are reported in Table 1.

In ventricular muscle fibers, procaine had little effect on the action potential duration, and benzocaine decreased it (Table 1). In Purkinje fibers from the same hearts, both anesthetics markedly decreased action potential duration (Table 1).

The Effect of Local Anesthetics in the Presence of Calcium

The finding that procaine and benzocaine had different effects on myocardial fiber contractile force (and also that there were exceptions to this rule) suggests that these agents have at least two opposing effects on force development and that one of these effects usually predominates. As far as the positive inotropic effect is concerned, it could be that local anesthetics affect the quantity of calcium released from the sarcoplasmic reticulum on excitation (Hatae, 1979). To gain some information concerning this point, procaine was given in the...
presence of normal and high calcium concentration to determine whether the increase in force caused by procaine is potentiated when the amount of calcium stored in the SR is increased. In Figure 2, procaine increased force in Tyrode’s solution (A). Increasing the calcium in the perfusing solution to 5.4 mM increased contractile force, as expected; however, adding procaine in the presence of high calcium increased the force to a smaller extent (B). Similar results were obtained in four experiments, as procaine increased the force by 36.2 ± 11.9% in Tyrode’s solution and by 9.5 ± 4.7% in high calcium solution. In one experiment, benzocaine decreased the force in the Tyrode’s solution and in the presence of high calcium (−70% and −60%, respectively).

The Effects of Local Anesthetics in the Presence of Norepinephrine

Procaine could increase the force by an adrenergic mechanism (Chiba, 1977), in agreement with the fact that it shifts the plateau to more positive values in ventricular fibers, as norepinephrine does. For this reason, procaine and benzocaine were administered first in the absence and then in the presence of norepinephrine.

In Figure 3, procaine increased the force by 11% in Tyrode’s solution (A). Norepinephrine increased the force, as expected, and procaine in the presence of norepinephrine increased the force by 128% (in contrast to 11% in control, B). In a Purkinje fiber preparation from the same heart, procaine decreased the force in the absence (−75%, C) and in the presence (−38%, D) of norepinephrine.

In six experiments, in ventricular muscle procaine (2.5 × 10⁻⁴ M) increased the force by 23.2 ± 22.4% in the absence and by 43.6 ± 13.4% in the presence of norepinephrine (10⁻⁷ M). The absolute increase in force by procaine was 13.9 ± 10.6 mg in the absence and 44.6 ± 14.3 mg in the presence of norepinephrine (P < 0.025).

In Purkinje fibers from four of the same hearts, procaine (2.5 × 10⁻⁴ M) decreased the force by 71.1

### Table 1 Effects of Local Anesthetics on Force and on Action Potential Duration

<table>
<thead>
<tr>
<th></th>
<th>Force (mg)</th>
<th>Δ%</th>
<th>P</th>
<th>APD (msec)</th>
<th>Δ%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular muscle fibers</td>
<td>17</td>
<td>47.1 ± 6.8</td>
<td>65.6 ± 12.7</td>
<td>Procaine</td>
<td>+39%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Purkinje fibers</td>
<td>16</td>
<td>52.4 ± 7.8</td>
<td>36.1 ± 6.8</td>
<td>Benzocaine</td>
<td>−34%</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>9.8 ± 1.5</td>
<td>1.6 ± 0.4</td>
<td>Procaine</td>
<td>−85%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>10.0 ± 1.7</td>
<td>0</td>
<td>Benzoicine</td>
<td>−100%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SE; n = number of experiments. Force (mg) = contractile force in mg. P = P value for paired Student’s t-test. APD (msec) = action potential duration measured in msec at 50% of the maximum amplitude. Δ% = percent changes in force or APD induced by local anesthetics. Control = values in the absence of local anesthetics. Procaine, Benzocaine = values obtained in the presence of procaine and benzocaine, respectively. The concentration of procaine was 2.5 × 10⁻⁴ M in all experiments; that of benzocaine was 5 × 10⁻⁴ M in 13 experiments and 2.5 × 10⁻⁴ M in three experiments in muscle and was 5 × 10⁻⁴ M in Purkinje fiber experiments.
± 7.4% in the absence and by 51.7 ± 8.1% in the presence of norepinephrine (5 × 10⁻⁷ M). Thus, it appears that the positive inotropic effect of procaine is potentiated in the presence of norepinephrine in muscle and the negative inotropic effect is decreased in Purkinje fibers.

The results obtained with benzocaine are illustrated in Figure 4. In ventricular fibers, benzocaine decreased the force of contraction in the absence (A) and increased force in the presence of norepinephrine (B). In a Purkinje fiber preparation from the same heart, benzocaine decreased the force of contraction in the absence (C) and in the presence of norepinephrine (D).

In eight experiments on ventricular muscle, benzocaine (5 × 10⁻⁴ M) decreased contractile force by 28.3 ± 10.8% in the absence and increased force by 32.6 ± 10% in the presence of norepinephrine (P < 0.01). In Purkinje fibers from six of these hearts, benzocaine (2.5 × 10⁻⁴ M) decreased force by 86.6 ± 2.9% in the absence and by 54.6 ± 8.0% in the presence of norepinephrine (P < 0.005).

**Local Anesthetics and β-Blockade**

The abolition of the potentiation between norepinephrine and procaine in ventricular muscle by propranolol is illustrated in Figure 5. Procaine actually decreased the force (A) and a similar effect occurred in the presence of high calcium (B). However, in the presence of norepinephrine, procaine increased contractile force (C). When the positive inotropic effect of norepinephrine was blocked largely by propranolol, procaine no longer increased force but instead decreased it (D), as it did in Tyrode’s solution.

In three experiments, procaine (2.5 × 10⁻⁴ M) decreased contractile force by 11.4 ± 11.5% in the absence and increased it by 21.5 ± 8.4% in the presence of norepinephrine. Propranolol (3.4 × 10⁻⁶ M) in these experiments reduced the force by 22.8 ± 0.2% (P < 0.05) and eliminated (+2.7%) the positive inotropic effect of norepinephrine (+65.6% in control): under these conditions, procaine decreased the force by 28.8 ± 2.8% (P < 0.025 with respect to the results obtained in the absence of propranolol).

In two experiments, benzocaine (5 × 10⁻⁴ M) decreased contractile force by 67.7% in the absence and increased it by 13.4% in the presence of norepinephrine. Propranolol (3.4 × 10⁻⁶ M) decreased the force by 37.2% and markedly reduced (+6%) the positive inotropic effect of norepinephrine (+97.7% in control): under these conditions benzocaine decreased the force by 73.1%.

Thus, propranolol abolished the potentiation between norepinephrine and local anesthetics and allowed the appearance of a negative inotropic effect.

**The Effect of Local Anesthetics in the Presence of Tetrodotoxin (TTX) and Norepinephrine**

If the positive inotropic effect of procaine and benzocaine involves an adrenergic mechanism, the negative inotropic effect could be due to depression of sodium influx. If this were the case, the local anesthetics could decrease force less or even in-
Figure 6  Modifications by TTX and norepinephrine of the inotropic effect of procaine and benzocaine in ventricular and Purkinje fibers. In A, the first panel shows the effect of TTX on force in ventricular muscle fibers; the second panel shows the increase in force induced by norepinephrine (NOREPL) administered at the vertical line. The third panel shows the effect of procaine (administered at the vertical line) in the presence of TTX and norepinephrine. The interruption between the first and second panels is 1 min—50 sec, and that between the second and third panel is 2 min—35 sec. In B, similar records as in A, for ventricular muscle fiber from a different heart in the presence of benzocaine. The interruption between the first two panels is 6 min—30 sec, and that between the second and the third panel is 4 min—20 sec. In C, are the changes in force in a Purkinje fiber preparation taken from the same heart as in A and subjected to the same procedure as in A. The interruption between the first and the second panels is 2 minutes and that between the second and third panel is 2 min—50 sec. In D, similar records as in B for a Purkinje fiber from the same heart as in B. The interruptions are 3 min—40 sec between the first and second panel and 1 min—35 sec between the second and third panel. The short break in the third panel was due to a failure of the fiber to respond to the stimulus which was increased in strength. Force calibration for each strip is at the end of the respective strip. Strip A of this figure and A and B of Figure 3 was from the same Purkinje preparation taken from the same heart as in B. The strips B and D of this figure were from the same preparations as in Figure 4.

Increase force in Purkinje fibers if the sodium influx is already decreased at the time local anesthetics are administered. This was tested by first decreasing sodium influx with TTX: as this results in a marked decrease in contractile force of Purkinje fiber bundles (Bhattacharyya and Vassalle, 1978) norepinephrine was added in the presence of TTX to reestablish contraction. Procaine and benzocaine then were added in the presence of TTX and norepinephrine. As shown in Figure 6, TTX decreased contractile force much less in ventricular muscle (A and B) than in Purkinje fibers (C and D), as expected. In both tissues, force increased when norepinephrine was administered in the presence of TTX. In ventricular muscle fibers, in the presence of both TTX and norepinephrine, procaine (A) and benzocaine (B) increased contractile force in a manner similar to that when norepinephrine alone was present (see Figs. 3 and 4). In Purkinje fibers, procaine (C) increased contractile force in the presence of TTX and norepinephrine, in contrast to the fall in force when norepinephrine alone was present (see Fig. 3). Benzocaine (D) still decreased contractile force in the presence of TTX and norepinephrine but much less than in the presence of norepinephrine alone (see Fig. 4).

Similar results were obtained in 10 experiments (Table 2). The average values reflect the general trend, although the differences were not always significant. It should be noted that in ventricular muscles, procaine and benzocaine increased force in the presence of norepinephrine alone by 52% and 44%, respectively: TTX little modified this effect (see Table 2). However, in Purkinje fibers, TTX did modify the effects of local anesthetics. Thus, in the presence of norepinephrine alone, procaine decreased the contractile force by 44% and benzocaine decreased the force more [-50%, \( P < 0.05 \) as compared to benzocaine effect in the presence of TTX and norepinephrine (see Table 2)].

The electrical events recorded from a Purkinje preparation are illustrated in Figure 7 (taken from the same experiments as in Figs. 4 and 6). In A, it is apparent that benzocaine in the presence of norepinephrine shortened the action potential markedly and reduced the force substantially. In B, TTX had reduced the duration of the action potential (−28%) and almost abolished the twitch (first

| Table 2  Effect of Local Anesthetics in the Presence of TTX and Norepinephrine (NE) |
|--------|----------------|----------------|----------------|
| n      | Force (mg)     | TTX            | TTX + NE       |
| Ventricular muscle fibers | | | |
| 4   | 33.7 ± 12.0 | 19.2 ± 7.6 | 52.9 ± 19.4 |
| 6   | 33.3 ± 6.1  | 21.1 ± 4.4  | 80.9 ± 16.5  |
| Purkinje fibers | | | |
| 4   | 11.2 ± 3.9  | 0.9 ± 0.5   | 15.4 ± 8.2   |
| 6   | 6.0 ± 1.6   | 0.5 ± 0.2   | 6.8 ± 2.2    |

Values are mean ± se. The concentrations of TTX was 5.3 x 10⁻⁴ M, of norepinephrine 5 x 10⁻⁷ M, and of procaine 2.5 x 10⁻⁴ M in all experiments and the concentration of benzocaine was 5 x 10⁻⁴ M in muscle and 2.5 x 10⁻⁴ M in Purkinje fiber experiments.
Panel). Norepinephrine shifted the plateau to more positive potentials, shortened the action potential, and increased the maximum diastolic potential (see second and third panels). Typically, adding benzocaine in the presence of TTX and norepinephrine decreased both the duration of the action potential and the magnitude of the contractile force very little (last B panel; contrast with the A panels). Thus, reduction of sodium influx by TTX little modifies the effects of local anesthetics in ventricular muscle but markedly reduces or even reverses the negative inotropic effect in Purkinje fiber bundles.

Local Anesthetics in the Presence of Veratridine

If the depressant effect of local anesthetics is reduced or abolished when these agents no longer can depress sodium influx, then increasing sodium influx prior to administration of local anesthetic should have an opposite effect. Veratrine is known to increase the sodium current during the upstroke and plateau and to increase force (Horackova and Vassort, 1973).

As shown in Figure 8A in a ventricular muscle preparation, veratridine increased the force of contraction. When procaine was added in the presence of veratridine, the force decreased by 47% [in contrast to a decrease by 75% in the absence of veratridine (see Fig. 3)].

Figure 9 shows the action potential and twitch of a ventricular muscle preparation. It is apparent that veratridine increased the amplitude and duration of the plateau and increased the force (second panel). It is also clear that benzocaine abolished both actions of veratridine (fourth and fifth panel).

In three experiments on Purkinje fibers, veratridine increased the duration of the action potential from 282.3 ± 26 to 332.6 ± 37 msec; benzocaine in the presence of veratridine decreased the action potential duration by 43%.

In nine experiments, veratridine increased the force of contraction in both ventricular muscle fibers and Purkinje fibers (Table 3). In ventricular muscle fibers, procaine increased the force of contraction in the absence and decreased it in the presence of veratridine (Table 3). In Purkinje fibers from the same hearts, procaine decreased the force in the absence and in the presence of veratridine [but more in its presence (Table 3)].
TABLE 3  Effects of Local Anesthetics in the Presence of Veratridine

<table>
<thead>
<tr>
<th></th>
<th>Ventricular muscle fibers</th>
<th>Purkinje fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Procaine</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Force (mg)</td>
<td>38.2 ± 6.9</td>
<td>58.5 ± 22.0</td>
</tr>
<tr>
<td></td>
<td>33.6 ± 13.0</td>
<td>48.7 ± 15.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>55.6 ± 13.3</td>
</tr>
<tr>
<td></td>
<td>31.4 ± 4.6</td>
<td>9.2 ± 1.7</td>
</tr>
</tbody>
</table>

|                | Benzocaine plus Procaine  | Benzocaine plus Benzocaine |
|                |                           |                             |
|                | 11.2 ± 4.0                | 2.8 ± 0.9                  |
|                | 9.9 ± 3.3                 | 25.0 ± 10.9               |
|                | 3.0 ± 1.4                 | (+152%)                   |
|                | 9.6 ± 1.9                 | 12 ± 0.4                  |
|                | 9.2 ± 1.7                 | 16.8 ± 3.0                |
|                | 1.0 ± 0.4                 | (+82%)                    |

Values are mean ± SE. The concentration of veratridine was 10⁻⁷ M and that of procaine 2.5 × 10⁻⁴ M in all experiments; the concentration of benzocaine was 5 × 10⁻⁴ M in muscle and 2.5 × 10⁻⁴ M in Purkinje fiber experiments.

* Paired Student’s t-test.

In ventricular muscle fibers, benzocaine decreased the force in the absence and more so in the presence of veratridine (Table 3). In Purkinje fibers, benzocaine decreased the force in the absence and more so in the presence of veratridine (Table 3).

Thus, in the presence of veratridine, procaine and benzocaine decreased the force of contraction in both ventricular and Purkinje fibers.

Discussion

Our results suggest that procaine and benzocaine: (1) increase the force of contraction through an adrenergic mechanism (this effect is more pronounced in muscle than in Purkinje fibers), and (2) decrease the force of contraction, possibly through a decrease of sodium influx (this effect is more pronounced in Purkinje fibers than in muscle). It also is apparent that (3) in muscle, procaine and benzocaine can cause either a positive or a negative inotropic effect but procaine more often causes a positive and benzocaine a negative inotropic effect, and that (4) the positive inotropic effect of these local anesthetics can be enhanced by prior reduction of Na influx (with TTX) and abolished by a prior enhancement of Na influx (with veratridine).

That both procaine and benzocaine have opposing effects on force in muscle was suggested by the fact that whereas, in general, procaine increased and benzocaine decreased force, there were exceptions with either anesthetic. This meant that whatever change in force was observed, it must have been the net result of opposing actions. The magnitude of each of these effects could conceivably vary in different preparations. That indeed more than one effect was exerted by either procaine or benzocaine was shown by the subsequent experiments in which either an increase or decrease in force could be induced with the same drug under suitable conditions.

A positive inotropic effect of procaine has been reported for canine atrial myocardium (Chiba, 1977) and bullfrog ventricular myocardium (Hatae, 1979). Both Chiba (1977) and Hatae (1979) considered the possibility that the increase in force might have been due to an adrenergic mechanism but came to different conclusions. Chiba showed that, with a concentration of propranolol that practically abolished the twitch, both norepinephrine and procaine had little effect on force. Chiba concluded that procaine probably had a direct stimulatory action on the β-adrenergic receptor. However, Hatae concluded that endogenous catecholamines are not responsible for the increase in force and that changes in inward calcium current or in calcium exchangeability were responsible for the procaine-induced increase in force. Thorens (1971) showed that higher concentrations of procaine (3.6 × 10⁻³ M) decreased contraction in cardiac muscle, possibly through marked depression of the resting and action potentials and conduction block. When depolarizing pulses were applied through a double-sucrose gap chamber, procaine did not decrease contraction. Baird and Hardman (1961) found that procaine did not modify the force of contraction of the turtle heart.

The present results are in agreement with those of Chiba (1977), possibly because we used tissues from the same species. That the positive inotropic effect of procaine may be mediated through some effect of β-receptor activation in muscle is supported by the findings that: (1) the plateau often was shifted to more positive potential values,
(2) norepinephrine potentiated the increase in force by procaine (and vice versa), and (3) the potentiation (and the positive inotropic effect) disappeared in the presence of propranolol. These findings support the concept that procaine has adrenergic effects, possibly by depressing norepinephrine uptake. Benzocaine has a weaker adrenergic effect which is shown by the reversal of the force effect in the presence of norepinephrine (Fig. 4). The adrenergic activity of local anesthetics appears less important in Purkinje fibers, but it can be demonstrated in the presence of norepinephrine when the negative inotropic effect of local anesthetics is prevented by the prior administration of TTX. In this situation, procaine often increases force (Fig. 6) and benzocaine decreases it less (Fig. 6).

The lack of potentiating effect by high calcium (as compared to norepinephrine) on the positive inotropic effect of procaine suggests that procaine does not release calcium from intracellular stores. Furthermore, the results suggest that procaine does not increase the slow inward current by a direct action but rather by enhancing the effect of norepinephrine on the β-receptor. Thus, it is norepinephrine that increases the slow inward current, not procaine. The lack of potentiation by high calcium may be accounted for by the fact that norepinephrine is less effective in increasing force when Ca\(^{2+}\) is high (see Morad and Roelett, 1972).

The negative inotropic effect of local anesthetics could be due to several possible mechanisms. One is that these drugs might reduce the slow inward current (Reiser et al., 1974; Josephson and Spere- lakis, 1976; Ducouret et al., 1979). This, however, conflicts with the finding that the slow response is little affected by local anesthetics (Carmeliet and Verdonck, 1974; Elharrar et al., 1977; Brennan et al., 1978). Furthermore, TTX [which also reduced force (Fig. 6)] does not reduce the slow inward current under voltage clamp conditions (Rougier et al., 1969), nor does it abolish the slow response depolarization of tissues (Carmeliet and Vereeceke, 1969; Pappano, 1970). Another possibility is that an impairment in the uptake or release of calcium from the cellular stores could play a role in the negative inotropic effect. If such interference with calcium movements into or out of cellular stores occurs in Purkinje fibers, it should not amount to a substantial block, because inotropic agents still act in the presence of local anesthetics (see Figs. 3 and 4; and Bhattacharyya and Vassalle, 1979).

A third possibility is that the mechanism of the negative inotropic effect of local anesthetics includes a fall in intracellular sodium and therefore in intracellular calcium. That local anesthetics decrease sodium influx has been shown in Purkinje fibers (Weidmann, 1955b; but separate studies with voltage clamp (Deck and Trautwein, 1964; Vassalle, 1966) and with TTX (Coraboeuf et al., 1979) suggest. Because both TTX (Coraboeuf et al., 1979) and local anesthetics (Carmeliet and Verdonck, 1974; present results) shorten the action potential of ventricular muscle fibers far less than that of Purkinje fibers, it seems reasonable to conclude that the sodium influx during the plateau is less in ventricular than in Purkinje fibers. A reduction of sodium influx during the plateau in Purkinje fibers by local anesthetics is supported by the findings of Carmeliet and Verdonck (1974), who demonstrated that aprindine increased the potential of Purkinje fibers depolarized at the plateau in zero potassium by reducing sodium influx rather than increasing K efflux. In the potential range negative to —40 mV, lidocaine also decreased the background sodium current and, in addition, increased the time-independent potassium current (Weld and Bigger, 1976). Similar effects at the plateau would contribute to shorten the action potential.

If the sodium influx at the plateau is decreased by local anesthetics, the question becomes: should, as a consequence, the contractile force decrease? If the action potential is shortened (due to the decreased sodium influx), the twitch may become smaller either because an early repolarization initiates relaxation sooner or because the calcium entry associated with sodium influx [sodium-calcium exchange (see Mullins, 1979)] is cut off sooner. This, however, does not seem to be the entire explanation of the negative inotropic effect of local anesthetics, for in the Purkinje fibers (where the fall in force is most pronounced) the twitch is over well before the end of the plateau. Because of this (see Fig. 1, C and D), it would matter little if the action potential became shorter. In fact, in the presence of local anesthetics (Fig. 1) or TTX (Fig. 7), the twitch may be practically abolished (rather than being reduced in amplitude or in duration). Also, local anesthetics shortened the action potential in the absence as well as in the presence of norepinephrine (see Fig. 7) but force decreased much less when norepinephrine was present. Finally, norepinephrine shortened the action potential in the presence of TTX (Fig. 7) and of local anesthetics but it induced or increased force development.

Although the shortening of the plateau as such does not seem to be important in the observed effects, the decreased sodium influx does. A decreased sodium influx should result in a decreased intracellular sodium concentration. A decreased intracellular sodium concentration should reduce the cellular calcium, as there is much evidence that intracellular sodium is related directly to intracellular calcium by means of the sodium-calcium exchange (see Blaustein, 1974; Langer, 1977; Mullins, 1979). The importance of a reduced sodium influx...
in the negative inotropic effect of local anesthetics is supported by: (1) the greater fall in force in Purkinje fibers which have a larger sodium influx (Coraboeuf et al., 1979) than in muscle fibers; (2) the elimination of or reduction in the negative inotropic effect in Purkinje fibers by reducing the sodium-dependent component of force before the administration of local anesthetics (by means of TTX administration); (3) the reduction of the negative inotropic effect in the presence of norepinephrine as the maintenance of the calcium store is assured by the larger slow inward current; and (4) the enhancement of the negative inotropic effect when the sodium influx is enhanced by the previous administration of veratridine. In regard to the last point, it should be noted that veratridine increases sodium influx by reducing the inactivation of the fast sodium current. Both the increase in sodium influx and force by veratridine are eliminated by TTX and in Na+-free solution, suggesting a causal relationship between the two events (Horackova and Vassort, 1973).

In conclusion, we propose that in ventricular muscle the maintenance of normal calcium stores is predominantly dependent on the slow inward current. Since the sodium entry during the plateau appears to be less than in Purkinje fibers (Coraboeuf et al., 1979), the cellular stores of calcium appear to be less affected by a curtailment of sodium influx. In Purkinje fibers, instead, the maintenance of cellular stores of calcium appears to be far more dependent on the larger sodium influx. Local anesthetics (as well as TTX) affect sodium influx in both types of fibers but, due to the larger steady state sodium current (Attwell et al., 1979), decrease the action potential duration and force much more in Purkinje fibers. When the maintenance of calcium stores is made more dependent on sodium influx in muscle (in the presence of veratridine) or in calcium influx in Purkinje fibers (in the presence of norepinephrine), the negative inotropic effect of local anesthetics became larger in the former and smaller in the latter tissue. Therefore, the different magnitude of the negative inotropic effect of local anesthetics in ventricular muscle fibers vs. Purkinje fibers appears to result from the different magnitude of the sodium influx rather than from a different mode of action.

References
Baird WM, Hardman HF (1961) An analysis of the effect of pH, procaine cation, non ionized procaine and procaine ethylchloride cation upon cardiac conduction time, stimulation threshold, amplitude of contraction and the relationship of these parameters to antiarrhythmic activity. J Pharmacol Exp Ther 132: 382-391
Brennan FJ, Cranefield PF, Wit AL (1978) Effects of lidocaine on slow response and depressed fast response action potentials of canine cardiac Purkinje fibers. J Pharmacol Exp Ther 204: 312-324
Carmeliet E, Vereecke J (1969) Adrenaline and plateau phase of the cardiac action potential. Pfluegers Arch 313: 300-315
Chiba S (1977) Procaine-induced positive chronotropic and inotropic responses of the isolated, blood-perfused dog atrium. Arch Int Pharmacodyn Ther 228: 104-115
Deck KA, Trautwein W (1964) Ionic currents in cardiac excitation. Pfluegers Arch 280: 63-80
Horackova M, Vassort G (1973) Ionic mechanism of inotropic effect of veratrine on frog heart. Pfluegers Arch 341: 281-284
Morad M, Rolett EL (1972) Relaxing effects of catecholamines on mammalian heart. J Physiol (Lond) 224: 537-558
Mullins LJ (1979) The generation of electric currents in cardiac fibers by Na/Ca exchange. Am J Physiol 236: C103-C110
Thorens S (1971) Electrical and mechanical effects of procaine on mammalian heart muscle. Pfluegers Arch 324: 56-66
Weidmann S (1958a) Effects of calcium ions and local anesthetics on electrical properties of Purkinje fibers. J Physiol (Lond) 129: 568-582
Weidmann S (1958b) The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. J Physiol (Lond) 127: 213-224
Local anesthetics and the role of sodium in the force development by canine ventricular muscle and purkinje fibers.

M Vassalle and M Bhattacharyya

Circ Res. 1980;47:666-674
doi: 10.1161/01.RES.47.5.666

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1980 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/47/5/666

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/