Effects of Calcium Channel Blockers on Pial Vascular Responses to Receptor Mediated Constrictors

WILLIAM I. ROSENBLUM

SUMMARY Published studies have seldom examined the in vivo effect of calcium channel blockers on the contractile response of cerebral vessels to receptor mediated constrictors, and have had little success in demonstrating any effect of a single systemic dose of the channel blockers in contrast to the effects of continuous infusions. The present study examines the effect of topical norepinephrine, prostaglandin F\textsubscript{2}\alpha and serotonin on pial arterioles of the mouse, in the presence of locally applied channel blockers and also 15 and 30 minutes after a single i.p. injection of the blockers. Verapamil, nisoldipine and nimodipine were all effective inhibitors of constriction by either route of administration, and in doses having little or no dilating action. The data not only indicate that single systemic doses can effectively alter contractile behavior of cerebral arterioles, but also demonstrate the importance of testing these drugs against receptor mediated constrictors whose effects, alone or in combination, may be important during initiation or maintenance of cerebral vasospasm.

FOR SEVERAL YEARS there has been particular interest in the actions of calcium channel blockers (CCB) on cerebral vessels, because of experimental evidence suggesting that cerebral vessels were especially sensitive to such agents.\textsuperscript{1-5} This sensitivity might permit CCB to improve cerebral blood flow and/or relieve cerebral vasospasm without alteration of circulation in other vascular beds. Some experimental studies published thus far use direct application of CCB to the cerebral vessels in vivo.\textsuperscript{6} Other studies utilize a continuous intravascular infusion of CCB.\textsuperscript{7-11} Studies of the effects of a single systemic dose on subsequent behavior of cerebral vessels appear rare, and in two such studies of which we are aware\textsuperscript{1,9} CCB failed to alter cerebral blood flow (CBF). It would seem important to investigate further, the action of single doses of systemically administered CCB on cerebral circulation. Moreover, in many vessels and species, in vitro, CCB may fail to influence resting vascular tone yet may markedly inhibit vasoconstriction.\textsuperscript{1,6,12} Therefore it is important to test the effect of CCB on constriction rather than simply test the effect on diameter or resting flow. The in vitro anticontractile effects of CCB on cerebral vessels appear particularly marked where receptor mediated agonists are the contractile agents.\textsuperscript{1,2}

Yet studies of cerebral circulation which report no effect of CCB on resting diameter\textsuperscript{3} or flow\textsuperscript{4,5} have not investigated the effects of CCB on receptor mediated constriction. Rather than agonists like serotonin, prostaglandin F\textsubscript{2}\alpha, or norepinephrine, hypocapnia or BaCl\textsubscript{2} have been used as the contractile stimulus.\textsuperscript{3-6} It seems advisable to study the effects of CCB on the contractile response to directly applied receptor mediated agonists. The following report describes such experiments, employing 3 different receptor mediated agonists, and 3 different CCB, each of the latter administered both directly to the pial vasculature and also given in a single intraperitoneal injection 15 and 30 minutes prior to testing the contractile response.

Methods

Male mice (Institute for Cancer Research Strain, Flow laboratories) weighing 22–35 g were anesthetized with urethan and subjected to tracheotomy and craniotomy as previously described.\textsuperscript{13,14} The dura was stripped as previously described\textsuperscript{13,14} and the cerebral surface (pial) vessels in the subarachnoid space between the transparent arachnoid and the brain were observed through a Leitz Ultraphak microscope.\textsuperscript{13,14} A TV camera and monitor were employed together with an image splitter and strip-chart recorder for measurement of diameter and diameter changes as described by Baez.\textsuperscript{15} In each mouse a single arteriole was arbitrarily selected for monitoring. The mice were maintained at 37°C, and the surface of the brain was irrigated with an artificial cerebrospinal fluid (CSF) flowing at 2 ml/min\textsuperscript{3} at 37°C and pH of 7.35 ± 0.03 (SD), as measured in the fluid passing across the craniotomy.
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site. The pH was maintained constant throughout an experiment.

Chemicals were applied to the cerebral surface as a bolus of 1.0 ml at 37°C delivered in 30 s. The chemicals used in this manner were: norepinephrine (Levodop bitartrate); serotonin creatinine phosphate (SHT, Sigma); prostaglandin F₂α; verapamil HCl (alpha-(3-((2-(3,4-dimethoxyphenyl)ethyl) - methylamino)propyl) - 3, 4-dimethoxy -(1-methylethyl) — benzeneacetonitrile; nimodipine (1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid methylpropyl ester or Bay k 5552); nisoldipine (1,4-dihydro-2,6-dimethyl -4-(2-nitrophenyl) -3,5-pyridine dicarboxylic acid methylpropyl ester or Bay k 5552). The verapamil was a gift from Knoll Pharmaceutical in studies utilizing either norepinephrine, 5HT or PGF₂α to constrict pial arterioles.

The verapamil was a gift from Knoll Pharmaceutical Co. Whippany NJ; the nimodipine and nisoldipine were gifts of Miles Laboratories, New Haven Ct. courtesy of Alexander Scriabine MD. The norepinephrine, SHT, PGF₂α, and verapamil were dissolved in the artificial CSF. The nimodipine and nisoldipine were dissolved initially in polyethyleneglycol (PEG) and then diluted in the artificial CSF. The quantity of the PEG in the final solution was either 100 or 10 parts per million. The pH of all solutions delivered to the brain surface was the same as that of the artificial CSF itself (7.35 ± .03). The action of the verapamil, and nimodipine was compared with that of the appropriate vehicle used in this manner were: norepinephrine (Levodop bitartrate); serotonin creatinine phosphate (SHT, Sigma); prostaglandin F₂α; verapamil HCl (alpha-(3-((2-(3,4-dimethoxyphenyl)ethyl) - methylamino)propyl) - 3, 4-dimethoxy -(1-methylethyl) — benzeneacetonitrile; nimodipine (1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylic acid methylpropyl ester or Bay k 5552). The verapamil was a gift from Knoll Pharmaceutical Co. Whippany NJ; the nimodipine and nisoldipine were gifts of Miles Laboratories, New Haven Ct. courtesy of Alexander Scriabine MD. The norepinephrine, SHT, PGF₂α, and verapamil were dissolved in the artificial CSF. The nimodipine and nisoldipine were dissolved initially in polyethyleneglycol (PEG) and then diluted in the artificial CSF. The quantity of the PEG in the final solution was either 100 or 10 parts per million. The pH of all solutions delivered to the brain surface was the same as that of the artificial CSF itself (7.35 ± .03). The action of the verapamil, and nimodipine was compared with that of the appropriate vehicle used in studies utilizing either norepinephrine, SHT or PGF₂α to constrict pial arterioles.

For the studies of calcium channel blockers (CCB) given intraperitoneally verapamil was dissolved in saline, while nimodipine and nisoldipine were dissolved first in PEG and then diluted with saline. Vehicle identical to that in the diluent was used to inject control mice which were alternated with drug treated mice. Separate studies were performed testing the responses of pial arterioles to either norepinephrine, 5-HT or PGF₂α. Each mouse was tested before the injection of CCB or vehicle, and then tested again fifteen and thirty minutes after the injection.

After each study blood gases and pH were determined on carotid artery blood with a micro blood gas analyzer (Radiometer). There were no effects of any of the drugs on arterial CO₂, O₂ or pH so these values are not presented with each study. Mean levels (± SD) for all experiments were CO₂ = 34 ± 2 mm Hg, O₂ = 95 ± 8, pH = 7.39 ± 0.04.

Results

Intraperitoneal Administration

The data is illustrated in table 1 which shows the inhibitory effect of a single injection on the contractile response to locally applied agonists. One way analysis of variance was used to compare constriction (expressed as % control diameter) 15 and 30 minutes after injection of CCB with constriction prior to injection. Initially 5 mg/kg verapamil was used and tested in 3 separate studies against 10 µg/ml NOR, SHT and PGF₂α respectively. In each case constriction was significantly inhibited. In 2 subsequent studies using lower doses of verapamil (0.5 and 0.01 mg/kg) it was

<table>
<thead>
<tr>
<th>Blocker and dose (mg/kg)</th>
<th>Constricting drug</th>
<th>Original diameter (µ)</th>
<th>% Constriction and (% Inhibition*</th>
<th>Before blocker</th>
<th>15 minutes after blocker</th>
<th>30 minutes after blocker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5.0</td>
<td>NOR</td>
<td>39 ± 5</td>
<td>15 ± 2</td>
<td>8 ± 3</td>
<td>46 ± 24</td>
<td>15 ± 3 (65 ± 20)</td>
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<td>5.0</td>
<td>SHT</td>
<td>38 ± 5</td>
<td>18 ± 3</td>
<td>10 ± 4</td>
<td>46 ± 7</td>
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<tr>
<td>5.0</td>
<td>PGF₂α</td>
<td>33 ± 4</td>
<td>21 ± 3</td>
<td>16 ± 5</td>
<td>26 ± 20</td>
<td>11 ± 3 (46 ± 14)</td>
</tr>
<tr>
<td>0.5</td>
<td>NOR</td>
<td>41 ± 3</td>
<td>21 ± 3</td>
<td>12 ± 5</td>
<td>41 ± 24</td>
<td>10 ± 4 (52 ± 18)</td>
</tr>
<tr>
<td>0.01</td>
<td>NOR</td>
<td>37 ± 3</td>
<td>21 ± 2</td>
<td>16 ± 6</td>
<td>23 ± 32</td>
<td>10 ± 2 (52 ± 13)</td>
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<tr>
<td>Nimodipine</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>NOR</td>
<td>35 ± 5</td>
<td>21 ± 5</td>
<td>12 ± 4</td>
<td>43 ± 7</td>
<td>29 ± 4 (57 ± 16)</td>
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<tr>
<td>0.001</td>
<td>NOR</td>
<td>35 ± 5</td>
<td>19 ± 8</td>
<td>13 ± 7</td>
<td>32 ± 25</td>
<td>9 ± 2 (61 ± 20)</td>
</tr>
<tr>
<td>0.001</td>
<td>PGF₂α</td>
<td>36 ± 3</td>
<td>19 ± 2</td>
<td>16 ± 3</td>
<td>13 ± 14</td>
<td>12 ± 5 (36 ± 23)</td>
</tr>
<tr>
<td>0.001</td>
<td>SHT</td>
<td>38 ± 4</td>
<td>17 ± 3</td>
<td>11 ± 10</td>
<td>31 ± 26</td>
<td>19 ± 9 (49 ± 17)</td>
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<tr>
<td>Nisoldipine</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>NOR</td>
<td>41 ± 6</td>
<td>15 ± 5</td>
<td>5 ± 2</td>
<td>64 ± 15</td>
<td>14 ± 3 (75 ± 18)</td>
</tr>
<tr>
<td>0.01</td>
<td>NOR</td>
<td>38 ± 2</td>
<td>19 ± 3</td>
<td>12 ± 7</td>
<td>38 ± 31</td>
<td>19 ± 4 (53 ± 23)</td>
</tr>
</tbody>
</table>

*% Constriction = (Original diameter − new diameter)/original diameter × 100; % Inhibition = (% Constriction before − % constriction after)/% constriction before × 100

†Blocker effect on % constriction significant (p < 0.05 ANOVA)

Each line in the table represents a single study employing 5 mice. The constrictor shown was applied topically to the pial vessels in a dose of 10 µg/ml and the calcium channel blocker than injected i.p. The response to the constrictor was retested 15 and 30 minutes later. The 3 responses to the constricting agent were compared using 1 way analysis of variance and in all but one study a significant diminution in the response (p < 0.05) was found following injection of the blocker.
decided to use only a single agonist in order to simplify the investigation, and NOR was arbitrarily chosen. Again constriction was significantly inhibited NOR, PGF$_{2\alpha}$ and SHT were then used in subsequent investigations of nimodipine. This CCB significantly reduced constriction to NOR at 0.01 mg/kg and to PGF$_{2\alpha}$ and SHT at 0.001 mg/kg. A final set of studies employed nimodipine. In order to simplify the investigation, and having shown that two other CCB were active against multiple agonists, we elected to use only NOR as the agonist in our studies of nimodipine. Again, constriction was significantly inhibited. In summary, eleven studies were performed with 3 CCB, and 3 agonists in varying combination, and in 10 of the 11 the CCB significantly reduced constriction as shown by analysis of variance comparing pre-treatment constriction with constriction 15 and 30 minutes after drug administration. When the diminution in constriction was expressed as a percent of the original constriction and labelled % inhibition we found that inhibition was slightly greater at 30 minutes after injection, compared with inhibition only 15 minutes after injection. These differences were not statistically significant in any given study because of the small number of animals (5) in each study, and because of the large standard deviation. However the same trend was seen in each of the 11 studies. Moreover, the difference between inhibition at 15 minutes and inhibition at 30 minutes was more pronounced at the lower doses of CCB, because at 15 minutes inhibition by the lower dose of CCB was less than that produced at 15 minutes by the higher dose. In none of the 11 studies was a significant change in arteriolar diameter observed following injection of CCB, any dilation being on the average 2 microns or less. In each of the 11 studies a parallel group of control mice (5 per study) was used. The control animals received vehicle rather than CCB, and the response to constrictor was observed 15 and 30 minutes later. In no study was there a significant diminution in response. Thus the decreasing contractile effect of the agonist cannot be due to either the passage of time or the effect of repeated application of the agonist.

**Topical Application**

Because no significant dilation was observed following intraperitoneal injection it was of interest to see whether local application of CCB could produce dilation. In each of 5 mice 3 doses of verapamil were applied to the pial surface for 30 seconds at 15 minute intervals followed by vehicle. Verapamil produced reversible dilations of $7 \pm 2, 11 \pm 4$ and $15 \pm 7\%$ at doses of 0.1, 1.0 and 5 $\mu g/ml$ (p = .06, ANOVA) while locally applied vehicle produced no dilation whatsoever ($0 \pm 0$).

In a similar study, nimodipine was used at a dose of 0.01 and 0.10 $\mu g/ml$. Dilation was observed only with 0.10 $\mu g/ml$ and was slight ($6 \pm 2\%$).

Since experiments with intraperitoneal CCB disclosed marked inhibition of constriction in the absence of dilation, it was of interest to determine whether topical doses of limited dilating capacity would also inhibit constriction. This is shown in table 2, which summarizes 4 separate studies. In each study, 5 mice were used and 10 $\mu g/ml$ constrictor was applied three times to the cerebral surface of each mouse at 15 minute intervals. On 2 of these occasions the constrictor was applied together with a CCB at the doses shown. On the third occasion only the constrictor plus vehicle was applied. In every case, the CCB displayed dose dependent inhibition of constriction (p < .05, ANOVA for each of the four studies.)

**Discussion**

The data clearly shows that the constriction of mouse pial arterioles, produced by receptor mediated agonists, is inhibited by simultaneous application of CCB. Verapamil was tested against NOR, SHT and PGF$_{2\alpha}$, and successfully inhibited, in a dose related fashion, the constriction produced by each agonist. Nimodipine was tested against NOR and inhibited constriction in a dose dependent fashion. Both verapamil and nimodipine were able to inhibit constriction at doses which did little or nothing to dilate pial arterioles as well as at doses which would dilate if presented to the vessels in the absence of the constricting agonist. The data clearly shows that a single intraperitoneal dose of verapamil, nimodipine or nisoldipine inhibited constriction produced by receptor mediated agonists locally applied 15 and 30 minutes after the injection of CCB. At the lowest dose of verapamil (0.01 mg/kg) there appeared to be less inhibition at 15 minutes than

<table>
<thead>
<tr>
<th>Blocker and dose</th>
<th>Original diameter (M)</th>
<th>Constrictor and dose</th>
<th>% Constriction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td>0.10</td>
<td>PGF$_{2\alpha}$ — 10</td>
<td>37 ± 4</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>PGF$_{2\alpha}$ — 10</td>
<td>37 ± 4</td>
</tr>
<tr>
<td></td>
<td>none</td>
<td>PGF$_{2\alpha}$ — 10</td>
<td>37 ± 4</td>
</tr>
</tbody>
</table>

*% Constriction = (Original diameter - new diameter)/original $\times 100$.

$p < 0.05$, ANOVA, 5 mice per study

Four experiments are displayed. In each experiment 5 mice were used. The constricting drug (10 $\mu g/ml$) was applied three times, 15 minutes apart, to the pial vessels of each mouse. On 2 of these applications, the calcium channel blocker was applied with the drug, in doses of 0.10 and 0.01 $\mu g/ml$. On the third occasion only constrictor and CCB vehicle were applied. In each study, a significant dose related inhibition of constriction was produced by the CCB (p < 0.05, ANOVA).
at 30 minutes, while at higher doses (0.5 and 5.0 mg/kg) the effects at 15 and 30 minutes were more equal. This suggests that lower doses take longer to achieve effective local concentrations than do higher doses. Similar observations were found when 2 doses of nimodipine (0.05 and 0.01 mg/kg) or 2 doses of nisoldipine (0.01 and 0.001 mg/kg) were compared. However, for each drug, great overlap in effectiveness of the various doses was observed.

The present data in a rodent (mouse) contrasts with the report of Altura, et al who failed to produce dilatation of rat pial arterioles with locally applied verapamil. We have no explanation for this discrepancy. However Altura et al did not test the capacity of verapamil to interfere with constriction produced by receptor mediated constrictors nor did they demonstrate that the pial arterioles were capable of dilating. Therefore it is possible that verapamil was being tested on maximally dilated vessels, and that an antagonistic effect on constriction could have been observed if it had been looked for.

Our studies with mice receiving intraperitoneal CCB is the first, to our knowledge, that uses a single injection rather than continuous infusion to inhibit constriction of pial arterioles. Our failure to alter diameter with i.p. injection resembles the failure of Edvinsson et al to alter cerebral blood flow in rats treated with a single i.v. injection and the failure by Harris et al and Harper et al to alter CBF after single i.v. injections in baboons. However none of these other studies tested the effect of CCB on receptor mediated constriction. Harper et al did not test constriction at all, while Edvinsson et al and Harris et al used only hypocapnia. Indeed, as pointed out in our introduction, even studies employing continuous infusion of CCB, have not tested the effect of the CCB against receptor mediated agonists, except possibly for the investigation of Haws and Heisted who did inhibit the contractile effect of CCB on receptor mediated constriction 1-2 of cerebral vessels. Indeed, as pointed out in our introduction, even studies employing continuous infusion of CCB, have not tested the effect of the CCB against receptor mediated agonists, except possibly for the investigation of Haws and Heisted who did inhibit the contractile effect of CCB on receptor mediated constriction 1-2 of cerebral vessels. Indeed, as pointed out in our introduction, even studies employing continuous infusion of CCB, have not tested the effect of the CCB against receptor mediated agonists, except possibly for the investigation of Haws and Heisted who did inhibit the contractile effect of CCB on receptor mediated constriction 1-2 of cerebral vessels. Indeed, as pointed out in our introduction, even studies employing continuous infusion of CCB, have not tested the effect of the CCB against receptor mediated agonists, except possibly for the investigation of Haws and Heisted who did inhibit the contractile effect of CCB on receptor mediated constriction

When interpreting our data, we were concerned about possible effects of lowered blood pressure (BP) on our results, since CCB can reduce BP. However, parallel studies of anesthetized mice injected with CCB failed to reveal a fall in BP 30 minutes after injection of the lowest effective doses of verapamil, nimodipine or nisoldipine used in this study. Moreover, we have previously shown that in mice, BP reduction is associated with enhanced rather than inhibited constriction. For these reasons, we believe the inhibition of receptor mediated constriction seen in the present study, was unrelated to alterations of BP.

Our data is important because of the in vitro literature claiming a greater effect of CCB on receptor mediated as opposed to potential mediatd constriction 1-2 of cerebral vessels and the literature showing an anticontractile effect of CCB in vitro 6, 12 at doses producing little or no change in basal tone. Our in vivo data is certainly consonant with that literature and would support the interest in the use of CCB for antispasmodic therapy of cerebral vessels whose constriction is thought to be produced by receptor mediated substances. On the other hand our data neither supports or denies a role for CCB in the dilation of normal or sclerotic vessels.

References


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