Effects of Calcium-Channel Blockers on Picrotoxin-Induced Centrogenic Arrhythmias in Cats

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SUMMARY

Intravenous picrotoxin injection has been established as a model of producing arrhythmias, mainly through enhanced central sympathetic outflow. The effects of calcium-channel blockers, and a beta-blocker on these arrhythmias were tested in chloralose-anesthetized cats. Picrotoxin (10 mg/kg, i.v.) produced mostly ventricular, sometimes supraventricular tachycardias and ectopic beats, as well as a marked elevation of arterial blood pressure. Nifedipine at the doses of 2 µg/kg (i.v. or i.c.) and 5 µg/kg (i.v.) transiently suppressed the arrhythmias in some of the cats tested. With the dose of 10 µg/kg (i.v.), it promptly and consistently abolished the arrhythmias without recurrence and significantly reduced the blood pressure (−62 ± 8/−59 ± 8 mmHg, Δ systolic pressure/Δ diastolic pressure, p < 0.001, n = 9). A similar degree of blood pressure reduction (−69 ± 8/−67 ± 7 mmHg, n = 6) after sodium nitroprusside (4–5 mg/kg, i.v.) injection abolished the arrhythmias in 4 of 6 cats; however, there was marked ECG evidence of myocardial ischemia in 3 cats. Verapamil (50 µg/kg, i.v.) transiently abolished the arrhythmias and significantly decreased the blood pressure (7/7 cats), whereas a larger dose (150 µg/kg) had a persistent effect (2/4 cats). Propranolol at a dose of 240 µg/kg also consistently abolished the arrhythmias without recurrence in all 4 cats.

We conclude that nifedipine, verapamil and propranolol are effective in the treatment of picrotoxin-induced arrhythmias. This result indicates that calcium-channel blockers or beta-blockers may be clinically effective in the treatment or prevention of arrhythmias caused by intracranial lesions with enhanced sympathetic outflow.
ICROTOXIN has long been used as a tool for eliciting cardiac arrhythmias through activation of the central nervous system.\(^{1-4}\) Although sympathetic and parasympathetic mechanisms are both implicated in this model, enhanced central sympathetic outflow to the heart and adrenally released catecholamines play a dominant role in the genesis of cardiac arrhythmias.\(^{2,3}\) The activation of adrenoceptors in coronary arteries and on cardiac cells provided a cellular basis for the genesis of arrhythmias. Alpha-adrenoceptor activation results in coronary vasoconstriction, which may cause cardiac ischemic changes and promote the occurrence of cardiac arrhythmias.\(^{4}\) Activation of alpha- and beta-adrenoceptors on cardiac cells produces cyclic-AMP-independent and cyclic-AMP-dependent increases in slow inward currents, respectively.\(^{5}\) Increases in slow inward currents may lead to the accumulation of intracellular calcium. Calcium overload plays an important role in the genesis of cardiac arrhythmias in several conditions, including increased catecholamine release.\(^{6-9}\) The release of calcium overload with calcium-channel blockers should block those types of arrhythmias. Therefore, experiments were performed to assess the effect of calcium-channel blockers on picrotoxin-induced arrhythmias.

**Materials and Methods**

Seventy-two cats of either sex weighing 1.6 to 3.3 kg were anesthetized with chloralose, 40 mg/kg, i.p. All cats were cannulated with endotracheal tubes and artificially ventilated with room air. The end expiratory CO\(_2\) concentration was maintained at 3.5–4.5%. They were immobilized with pancuronium bromide, initially with 0.08 mg/kg followed by 0.02 mg/kg i.v. every 20–40 min. The femoral vein and artery were catheterized for drug administration and blood pressure monitoring, respectively. In some experiments polyethylene tubes (Intramedic) were inserted into the vertebral artery or advanced via the left femoral artery through the aorta into the left ventricle for intravertebral or intracardiac injections of nifedipine. The blood pressure was monitored with a Gould P23ID transducer connected to an amplifier. Heart rate was monitored with a Gould Biotech triggered by arterial pulses. The lead II electrocardiogram (ECG) was monitored by a Gould universal preamplifier and displayed continuously on a Tektronix 922 oscilloscope.
Heart rate, blood pressure and ECG were recorded continuously with a Gould ES-1000 recorder.

Picrotoxin (Sigma Chemical Co.) was dissolved in 10% ethanol in normal saline. Nifedipine (Towarat, Towa Co.) was dissolved in 40% polyethylene glycol and the final concentration was 10 µg/ml. Both drugs were freshly prepared on the experimental day. The containers for these drugs were wrapped in aluminum foil. All lights in the room were turned off during injection. Picrotoxin was given by intravenous bolus injection at a dose of 10 mg/kg to induce cardiac arrhythmias. Nifedipine, verapamil (Orion Pharmaceutica), propranolol (Sigma) and sodium nitroprusside (Nipride, Roche) were administered within 3 min after the onset of arrhythmias.

Data are expressed as means±SEM. Comparisons were made with paired Student’s t-test or Mann-Whitney Rank Sum Test. A p value less than 0.05 was regarded as statistically significant.

RESULTS

Data were obtained from 66 cats which developed tachycardias or ectopic beats after picrotoxin injection. The remaining 6 cats were excluded due to the absence of the above-mentioned arrhythmias.

After injection of picrotoxin there were dramatic changes in heart rate and blood pressure. In 1 min, the heart rate slowed from 203±5 to 172±5 beats/min (−31±3 beats/min, p<0.001, n=66). The blood pressure decreased concurrently from 154±3/115±3 mmHg to 134±4/93±3 mmHg (−20±3/−22±2 mmHg, p<0.001, n=66). Following this initial decrease,

<table>
<thead>
<tr>
<th>Arrhythmias</th>
<th>Nifedipine (10 µg/kg)</th>
<th>Verapamil (50 µg/kg)</th>
<th>Nitroprusside (4-5 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>1. Monofocal VEB</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2. Multifocal and paired VEB</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>3. Ventricular tachycardia</td>
<td>7</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>4. Supraventricular ectopic beats</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5. Junctional tachycardia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6. Sinus rhythm</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>7. Junctional rhythm</td>
<td>0</td>
<td>2**</td>
<td>0</td>
</tr>
</tbody>
</table>

“n” denotes number of experimental animals. VEB=ventricular ectopic beat.
* Ventricular tachycardia recurred in 1 animal at 13 min after nitroprusside.
** Converted to sinus rhythm at 3 and 7 min after nifedipine, respectively.
the blood pressure increased to a peak of 245±5/160±3 mmHg (+90±5/
+45±3 mmHg, p<0.001) within 4 min. Associated with the elevation of
blood pressure, the heart rate slowed further to 132±5 beats/min (−63±6
beats/min as compared with the level before picrotoxin injection, p<0.001,
n=66). Cardiac arrhythmias developed immediately when the blood pres-
sure peaked. They were mostly ventricular and sometimes supraventricular
tachycardias and/or ectopic beats (Table I). Records from a typical experi-
ment are shown in Fig. 1.

The duration of arrhythmias induced by a single picrotoxin injection was
43±11 min (from 15 to 65 min) in 4 cats given no other drugs.

Effects of nifedipine and verapamil on picrotoxin-induced arrhythmias:

The effects of intravenous nifedipine at doses of 2, 5 and 10 µg/kg were
tested in 19 cats. At smaller doses (2 and 5 µg/kg), nifedipine only temporar-
ily (for 4 to 30 min) suppressed the arrhythmias in 6 of 10 cats (Table II). At a
dose of 10 µg/kg, it converted the arrhythmias to sinus rhythm within 30 sec
in 7 cats and to junctional rhythm, then to sinus rhythm in 2 cats (Tables I
and II). Arrhythmias did not recur within an observation period of at least
45 min.

After injection of picrotoxin ventricular ectopic beats developed within
5 min (Fig. 1). Nifedipine (10 µg/kg bolus) was injected at 7 min 30 sec (i.e.
2 min 30 sec after the occurrence of arrhythmias). About 15 sec later the
ECG showed normal sinus rhythm. The arrhythmias did not recur during
more than 1 hour of observation afterwards. Note that the blood pressure
dropped progressively after nifedipine injection and stabilized 1 min later.
Injection of 10 µg/kg nifedipine reduced the blood pressure (−62±8/−59±
8 mmHg) and heart rate (−39±16 beats/min) significantly (Fig. 1 and Table
III).

To achieve a primarily local effect on the heart, intracardiac injection of
2 µg/kg nifedipine was performed in 6 cats. Arrhythmias were also abolished,
but after 7±2 min the arrhythmias recurred. The blood pressure decreased

Table II. Effects of Nifedipine by Intracardiac (i.c.) and Intravenous (i.v.) Routes
with Various Dosages on Picrotoxin-Induced Arrhythmias

<table>
<thead>
<tr>
<th>Route and dosage</th>
<th>2 µg/kg, i.c. (n=9)</th>
<th>2 µg/kg, i.v. (n=5)</th>
<th>5 µg/kg, i.v. (n=3)</th>
<th>10 µg/kg, i.v. (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Ineffective</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Duration of effects</td>
<td>6.7±2.3 min</td>
<td>*</td>
<td>**</td>
<td>***</td>
</tr>
</tbody>
</table>

* Lasting 20 sec, 4 min and 10 min, respectively.
** Lasting 10 min, 11 min 30 sec and 30 min, respectively.
*** Lasting more than 60 min in 7 animals, and 21 min and 29 min, respectively, in 2 other animals.
Fig. 1. Effects of nifedipine on picrotoxin-induced arrhythmias. Left columns show the slow-speed simultaneous recordings of the lead II electrocardiogram (ECG, upper traces), heart rate (HR, middle traces) and blood pressure (BP, lower traces) in sequence. The letters a–e above the slow-speed traces represent the time at which the corresponding fast-speed ECGs in right column were recorded. The time indicated above the letters b–e is the interval in reference to the injection of picrotoxin (P, arrow). Nifedipine (N, arrow) was given at 2 min 30 sec after the onset of arrhythmias. Note that arrhythmias were abolished abruptly 15–20 sec after the nifedipine injection. Also note that elimination of arrhythmias occurred before a marked lowering of blood pressure.

from 227±9/162±6 mmHg to 178±16/101±10 mmHg (-48±18/-61±16 mmHg, p<0.05 for systolic and p<0.02 for diastolic, respectively).

Intravertebral injection of nifedipine was performed in 7 cats to examine if its local effect in the brain could terminate the arrhythmias. Nifedipine at the dose of 1 µg/kg terminated the arrhythmias in only 1 cat. When the dose was increased to 2 µg/kg it abolished the arrhythmias in 3 of 6 cats. In the 4 effective cases, the arrhythmias were abolished 43±18 sec (10–90 sec) after nifedipine injection and the sinus rhythm was maintained for only 5±1 min. The arrhythmias then recurred. The blood pressure declined gradually and stabilized at 194±12/127±9 mmHg (-44±7/-39±9 mmHg, p<0.001 for systolic and p<0.01 for diastolic, respectively, n=7) at 84±13 sec (n=7, 50–120 sec) after nifedipine injection.

Intravenous injection of verapamil at the dose of 10 µg/kg had no antiarrhythmic effects in 5 cats. However, a dose of 50 µg/kg consistently converted
Table III. Effects of Administration of Various Antiarrhythmic Agents on the Blood Pressure and Heart Rate of Cats Receiving Picrotoxin

<table>
<thead>
<tr>
<th></th>
<th>Nifedipine (10 µg/kg) n=9</th>
<th>Verapamil (50 µg/kg) n=7</th>
<th>Propranolol (240 µg/kg) n=4</th>
<th>Nitroprusside (4-5 mg/kg) n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>187±9</td>
<td>244±10</td>
<td>230±9</td>
<td>217±19</td>
</tr>
<tr>
<td>A</td>
<td>126±7</td>
<td>211±9</td>
<td>189±26</td>
<td>148±16</td>
</tr>
<tr>
<td>D</td>
<td>-62±8***</td>
<td>-33±4***</td>
<td>-44±26</td>
<td>-69±8***</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>138±8</td>
<td>169±6</td>
<td>161±4</td>
<td>158±15</td>
</tr>
<tr>
<td>A</td>
<td>80±5</td>
<td>145±6</td>
<td>131±14</td>
<td>91±18</td>
</tr>
<tr>
<td>D</td>
<td>-59±8***</td>
<td>-24±5**</td>
<td>-30±16</td>
<td>-67±7***</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>189±15</td>
<td>176±11</td>
<td>211±19</td>
<td>169±14</td>
</tr>
<tr>
<td>A</td>
<td>132±9</td>
<td>139±6</td>
<td>144±7</td>
<td>124±15</td>
</tr>
<tr>
<td>D</td>
<td>-39±16*</td>
<td>-37±9**</td>
<td>-67±20*</td>
<td>-46±9**</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01, *** p<0.001.
B and A denote data before and after drug administration, respectively. D denotes the difference between B and A.
SBP=systolic blood pressure; DBP=diastolic blood pressure; HR=heart rate; n=number of experimental animals.
Note that all the values shown were collected at stabilized maximal changes after administration of respective agents.

Fig. 2. Effects of verapamil (panel A), nitroprusside (panel B) and propranolol (panel C) on picrotoxin-induced arrhythmias. In each panel the upper trace is the lead II electrocardiogram and the lower trace is blood pressure recording at two speeds. The drugs were injected intravenously as a bolus (time indicated by arrows). Verap=verapamil; NP=nitroprusside; PPL=propranolol.
the arrhythmias to sinus rhythm (Table I) and significantly decreased the blood pressure and heart rate (Table III) in 7 cats. However, $14 \pm 4$ min ($n=7$) later, the arrhythmias resumed; the blood pressure remained unchanged. A larger dose of verapamil (150 $\mu$g/kg) abolished the arrhythmias for a longer duration in all 4 animals tested. However, suppression for 1 hour of observation was only seen in 2 cats (Fig. 2A). Note that the arrhythmias were abolished at 40 sec and the blood pressure was maximally reduced 1 min after verapamil injection.

Effects of Propranolol on picrotoxin-induced arrhythmias:

Propranolol at doses of 20–120 $\mu$g/kg transiently suppressed the picrotoxin-induced arrhythmias in 4 cats. At a larger dose (240 $\mu$g/kg), it consistently and persistently abolished the arrhythmias (Table I). The abolition occurred 30 sec after initiation of propranolol (240 $\mu$g/kg) injection, before the blood pressure changed significantly (from $230 \pm 18/161 \pm 9$ mmHg to $233 \pm 15/154 \pm 12$ mmHg, $n=4$). However, the blood pressure decreased progressively and stabilized at $155 \pm 59/115 \pm 37$ mmHg 2 to 3 min after propranolol injection (Table III and Fig. 2C).

Effects of nitroprusside on picrotoxin-induced arrhythmias:

To evaluate the role of blood pressure reduction caused by calcium-channel blockers in the antiarrhythmic action, sodium nitroprusside was tested in 6 cats. Intravenous injection of nitroprusside (4–5 mg/kg) reduced the blood pressure markedly ($-69 \pm 8/-67 \pm 7$ mmHg) within 1 min (Table III). This degree of blood pressure reduction was similar to that induced by 10 $\mu$g/kg nifedipine ($p>0.05$ by Mann-Whitney Rank Sum Test). Nitroprusside indeed converted the picrotoxin-induced arrhythmias to sinus rhythm in 4 cats (Table I and Fig. 2B), although ventricular arrhythmias recurred in 1 cat. In contrast to nifedipine, nitroprusside induced marked ST-T wave changes suggesting myocardial ischemia in 3 cats following abolition of the arrhythmias (Fig. 2B). In the 4 effective cases, the blood pressures were maximally reduced at $65 \pm 9$ sec and the arrhythmias were abolished at $133 \pm 28$ sec after nitroprusside injection (Fig. 2B).

Discussion

In the present experiment, the arrhythmias induced by i.v. picrotoxin were effectively terminated by calcium-channel blockers (nifedipine and verapamil), a beta-adrenergic blocker (propranolol) and a vasodilator (sodium nitroprusside). These arrhythmias are neurogenic, arising mainly through enhanced sympathetic activity.$^{1-4}$ Thus, the present findings suggest that, except for sodium nitroprusside (which abolished the arrhythmias at the ex-
pense of resulting cardiac ischemia), these drugs are potentially effective in other neurogenic arrhythmias in acute stroke patients.\textsuperscript{10,11} The arrhythmias in these patients may also be due to increased sympathetic activity, since the plasma catecholamine level in the stroke patients was higher than the control subjects.\textsuperscript{12}

The major finding in the present study is that calcium-channel blockers, such as nifedipine and verapamil are effective therapy for neurogenic arrhythmias which may be due to sympathetic excitation or the release of catecholamines. This effect is presumably ascribed to calcium regulation of cardiac cells and/or to coronary vasodilatation resulting in relief of myocardial ischemia.\textsuperscript{13} The importance of a local action of nifedipine on the heart has been demonstrated by the following experiments. First, nifedipine through intracardiac injection at a small dose of 2 μg/kg was shown to abolish picrotoxin-induced arrhythmias in all 6 animals tested, but the effect was transient (Table II). The transient effect may be explained by its ready dilution after injection. Second, at the same or higher dose (5 μg/kg) via an intravenous route nifedipine was only partially effective (Table II). Third, an intravenous dose of 10 μg/kg was needed to achieve a complete and prolonged abolition of the arrhythmias (Table II).

On the other hand, nifedipine may act centrally to cause bradycardia and hypotension,\textsuperscript{14} which may contribute to the antiarrhythmic effect. However, the same small dose (2 μg/kg) of nifedipine through both intravertebral and intravenous administrations only eliminated these arrhythmias transiently and partially (Table II), suggesting a relatively weak central action.

Furthermore, one may argue that the reduction of markedly elevated blood pressure could contribute to the abolition of these arrhythmias and/or the maintenance of their antiarrhythmic effects. This is supported by the fact that sodium nitroprusside, which reduced the blood pressure in a degree similar to nifedipine, also abolished these arrhythmias in some animals (Fig. 2B). However, the antiarrhythmic effects of calcium-channel blockers may not depend on this hypotensive action because nifedipine (Fig. 1) and verapamil (Fig. 2A) abolished the arrhythmias shortly after administration, but before the blood pressure was markedly lowered. Thus, both the local cardiac effects and the hypotensive effect may account for the antiarrhythmic action of these calcium-channel blockers. It is important to note that although the arrhythmias were terminated by nitroprusside in 4 of 6 cats, residual myocardial ischemia occurred after termination of the arrhythmias in 3 of 4 cats. Therefore, nitroprusside is not an ideal agent in these arrhythmias. The residual myocardial ischemia could be a result of shunting the coronary blood flow away from ischemic zones.\textsuperscript{15}
The duration of the verapamil effect in suppressing arrhythmias was transient at the dose of 50 μg/kg and persistent at the dose of 150 μg/kg in 2 of 4 cats. The short duration of action could be due to the more rapid distribution to tissues following injection as compared to nifedipine.

It was shown by DiMicco et al. that picrotoxin-induced arrhythmias could be prevented by an alpha- but not by a beta-adrenergic blocking agent. In the present experiment, propranolol at a dose of 240 μg/kg has consistent antiarrhythmic effects, confirming the antiarrhythmic effect of propranolol in another model of neurogenic arrhythmia. The contradictory result of propranolol in the experiment by DiMicco et al. can hardly be accounted for by the differences between drug dosages (1 mg/kg vs. 240 μg/kg) or the modes of drug administration (given after vs. before the onset of arrhythmias).

Lee et al. reported that cardiac arrhythmias induced by intravenous injection of picrotoxin (4 mg/kg) could last 15–24 min (mean 18 min). In the present study, for the sake of inducing consistent and prolonged arrhythmias, the dose of picrotoxin was increased to 10 mg/kg. The induced arrhythmias lasted 15–65 min in 4 control cases. In all experiments, antiarrhythmic agents were given within 3 min after the onset of arrhythmias and the arrhythmias subsided consistently and abruptly within 30 sec in the cats receiving a sufficient dose of nifedipine, verapamil or propranolol. Therefore, a spontaneous conversion seems implausible.

Acknowledgments

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