The Antiarrhythmic Effect of Systemically Administered Moxonidine, a Selective Imidazoline (I), Receptor Agonist, is Mediated by a Central Mechanism in Rats

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Abstract: Background: Moxonidine, a selective imidazoline, (I,) receptor agonist, possesses antiarrhythmic properties. The goal of the present study was to determine whether the antiarrhythmic effects of systemically administered moxonidine are exerted via central or peripheral I, receptors in rats with halothane-adrenaline-induced arrhythmias. Method: Rats were anesthetized with halothane and systemic arterial pressure and heart rhythms were continuously monitored. The arrhythmogenic dose of adrenaline was defined as the smallest dose that produced three or more premature ventricular contractions within 15 sec period. Results: Systemically administered moxonidine dose-dependently inhibited adrenaline-induced arrhythmias. Intracerebral efavroxn (a selective I, antagonist with affinity for a, adrenoceptors) but not rauwolscine (an a, antagonist without affinity for imidazoline receptors) blocked the antiarrhythmic effect of moxonidine. Furthermore, the antiarrhythmic effect of moxonidine was completely abolished in both vagotonized and atroline methylnitrate-treated rats. Conclusion: Systemically administered moxonidine prevents the halothane-adrenaline-induced arrhythmias through activation of central, but not peripheral, I, receptors.

Key words: Imidazoline receptor, moxonidine, arrhythmias, central nervous system

INTRODUCTION

Previous studies suggest that some of the cellular effects of the a, agonist, clonidine, were mediated via non-adrenergic imidazoline receptors (Bousquet et al., 1984; Tibirica et al., 1991), which can be sub-classified into imidazoline, receptors (I,) and imidazoline, receptors (I,). Berdeu et al., 1995; Parini et al., 1996). Our previous reports documented that the selective I, subtype receptor agonist, rilmenidine (Parini et al., 1996), prevented halothane-epinephrine arrhythmias via modulation of central imidazoline receptors (Mamamoto et al., 1996, Takada et al., 1997).

Moxonidine is more selective agonist for the I, receptor (Ernsberger et al., 1993) and has been studied in clinical trials (Swedberg et al., 2002). Experiments in animal studies have demonstrated that moxonidine exerted antiarrhythmic effects (Lepran and Papp, 1994; Poisson et al., 2000) and I, receptors in the central nervous system is involved in the antiarrhythmic effect of moxonidine (Kagawa et al., 2005). Previous investigations have reported that the I, receptor was expressed in cardiac tissue (El-Ayoubi et al., 2002) and that moxonidine modulated norepinephrine release by binding to presynaptic receptors (Schäfer et al., 2002). Thus, it may unclear whether the antiarrhythmic effects of moxonidine are mediated by a central or peripheral mechanism, when it is systemically given. The goal of the present study was to determine whether the central mechanism is involved in the antiarrhythmic effects of systemically administered moxonidine in rats using halothane-adrenaline-induced arrhythmias.

MATERIALS AND METHODS

All protocols were approved by the Animal Care Committee of Osaka University Faculty of Medicine. Male Sprague-Dawley rats, weighing 360-430 g were used and housed in groups of four in temperature-controlled environment under 12-h light:12-h dark cycles with free access to food and water. The animals were anaesthetized with 2% halothane in oxygen. After tracheotomy, the lungs were mechanically ventilated with a tidal volume of 12 mL/kg⁻¹ at 40-50 breaths min⁻¹ (Rodent Ventilator; Ugo Basile, Vasile, Italy). The respiratory rates were adjusted to maintain PaCO₂ at 40±5 mm Hg. Then, the inspired
concentration of halothane was adjusted to 1.5% with an
anesthetic gas analyzer (Eapnomae ultima multiple gas
monitor, Datex, Helsinki, Finland). Lead II of the
Electrocardiogram (ECG) and heart rate was monitored
continuously by ECG amplifier and pulse counter unit
(AC-611G; Nihon Kohden, Tokyo, Japan). A polyethylene
catheter (PE-50, PE-10) was inserted into the femoral artery
for blood sampling and pressure monitoring with a
pressure transducer unit (AP-641G; Nihon Kohden) and
another catheter was inserted into the femoral vein for
administration of drugs. The ECG and arterial blood
pressure were recorded continuously with a thermal array
recorder (WS-641G; Nihon Kohden). A heating pad was
used to maintain rectal temperature at 38.0±0.5°C. Arterial
pH and oxygen tension was maintained at 7.40±0.05 and
more than 100 mm Hg, respectively. After completion of
preparation, anesthesia was maintained for 30 min to
achieve a steady state before experiments commenced.

The arrhythmic dose of adrenaline was defined as the
dose that produced three or more premature
ventricular contractions within 15 sec of injection.
According to a previous report, epinephrine was injected
at logarithmically-spaced doses (0.5, 0.71, 1.0, 1.41, 2.20,
2.83, 4.0, 5.67, 8.0, 11.4 µg kg⁻¹) following an initial dose
of 4.0 µg kg⁻¹ (Takada et al., 1993). The 4.0 µg kg⁻¹ dose
of adrenaline was used as initial dose; if premature
ventricular contractions were absent, the dose was
sequentially increased and if premature ventricular
contractions were induced by this initial dose, the dose
was sequentially decreased. This method minimized the
number of adrenaline injections necessary to determine
arrhythmic dose. A period of 10-30 min was allowed
between each injection to allow stabilization of
hemodynamic parameters (arterial blood pressure and
heart rate).

When the criterion for arrhythmic dose was satisfied, a 2.0 mL arterial blood sample was collected for
the measurement of the plasma concentration of
adrenaline. The blood samples were placed into
pre-cooled plastic tubes containing 20 µL of 0.2 M
EDTA-2Na and 0.2 M Na₂SO₄, and then centrifuged at
4,000 rpm for 10 min at 2°C to separate the plasma. For
analysis of adrenaline, 0.5 mL of plasma was acidified by
the addition of 0.25 mL of 2.5% perchloric acid to
precipitate protein. The samples were stored at -40°C until
analysis (no longer than 7 days). The plasma
concentration of adrenaline was determined by a fully
automated high-performance liquid chromatography-
fluorimetric system (HLC-8030 Catecholamine Analyzer;
Tosoh, Tokyo, Japan) using the diphenylenethyleneamine
condensation method (Nohta et al., 1984). This assay
method has a limit of sensitivity of 10 pg mL⁻¹ for
adrenaline and the inter- and intra-assay variation were
both less than 3%.

Effect of systemic moxonidine on halothane-adrenaline-
induced arrhythmias: The arrhythmic doses and plasma
concentration of adrenaline were determined in the
presence of moxonidine (0, 20, 40, 60 µg kg⁻¹). Moxonidine
or vehicle was administered intravenously (IV) and then adrenaline was administered 30 min later. In
order to characterize the receptor mechanism of the effect
of moxonidine, the arrhythmic doses and plasma
concentration of adrenaline were determined in the
presence of moxonidine (60 µg kg⁻¹, IV) with intracisternal
(IC) vehicle (10 µL), rauwolscine (40 µg kg⁻¹, IC), or
efaroxan (20 µg kg⁻¹, IC), respectively. Rauwolscine is a classical α₂ adrenoceptor antagonist with no affinity for
imidazoline receptors (Lehmann et al., 1989) and efaroxan
is an α₂ adrenoceptor antagonist with high affinity for I₁
receptors and little affinity for I₂ subtype (Lione et al.,
1998). In each rat, a 30-G stainless steel needle was placed
into the cisterna magna through the atlanto-occipital
membrane in order to administer these antagonists.
The correct position of the cannula was checked by the efflux
of clear cerebrospinal fluid. Doses of rauwolscine and
efaroxan were determined to be approximately equal with
regard to α₂ adrenoceptors blocking efficacy (Campbell
and Potter, 1995). In these groups, the intracisternal
injection of the antagonists was performed 15 min before
intravenous moxonidine.

Influence of bilateral vagotomy on the effects of
moxonidine: The arrhythmic doses and plasma
concentration of adrenaline were determined with or
without moxonidine (60 µg kg⁻¹, IV) in the bilaterally
vagotomized animals. Bilateral vagotomy was performed
by sectioning both vagus nerves at the level of the fourth
cervical vertebra. In addition, because the vagus nerve is
composed of afferent and efferent fibers, the effect of
moxonidine (60 µg kg⁻¹, IV) were examined in the presence
of atropine methylnitrate (5.0 mg kg⁻¹, IV), which can
block efferent vagal outflow to the heart (Langhans et al.,
1985; Langhans and Scharrer, 1987). In this study,
atropine methylnitrate was given intravenously 15 min
before moxonidine administration.

Drugs: Moxonidine was kindly provided by Lilly
Research Laboratories (Indianapolis, IN). Other chemicals
were obtained from the sources indicated: halothane
(Takeda Chemical, Osaka, Japan), (+)-adrenaline (Wako
Chemical, Osaka, Japan), efaroxan and rauwolscine ( RBI,
MA, U.S.A.), atropine methylnitrate (Sigma Chemical, MO,
U.S.A.). Moxonidine was dissolved in 0.1 mL acetic
acid (0.01 M) and diluted with physiological saline (0.9% W/V% sodium chloride, Terumo, Tokyo, Japan) to the desired concentration, such that each rat received doses as 0.2 mL bolus injections. Efaroxan and rauwolscine were dissolved in saline to the desired concentration, such that each rat received doses in volumes of 10 μL. Adrenaline was dissolved in 0.1 mL HCl (1N) and diluted with saline to the desired concentration, such that each rat received doses as 0.2 mL bolus injections.

**Data analysis:** All data are expressed as Mean±SD. Data were analyzed by one-way analysis of variance and comparisons between groups were assessed by Scheffe’s test *p<0.05* was considered statistically significant.

**RESULTS**

Moxonidine (0, 20, 40, 60 μg kg⁻¹, IV) increased the arrhythmogenic dose and the plasma concentration of adrenaline in a dose-dependent manner (Fig. 1).

Haemodynamic data obtained at the onset of arrhythmias were not significantly different when comparing the different moxonidine groups (Table 1).

Central administration of efaroxan (20 μg kg⁻¹, IC) completely blocked the antiarrhythmic effect of moxonidine (60 μg kg⁻¹, IV). By contrast, central administration of rauwolscine (40 μg kg⁻¹, IC) did not significantly alter the effect of moxonidine (60 μg kg⁻¹, IV) (Fig. 2).

**Table 1:** Haemodynamic data at the onset of arrhythmias in the presence of moxonidine during halothane anesthesia

<table>
<thead>
<tr>
<th>Dose of moxonidine (μg kg⁻¹, IV)</th>
<th>n</th>
<th>SAP (mm Hg)</th>
<th>DAP (mm Hg)</th>
<th>HR (beats min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>13±18</td>
<td>86±17</td>
<td>41±47</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>129±12</td>
<td>86±11</td>
<td>368±47</td>
</tr>
<tr>
<td>40</td>
<td>8</td>
<td>14±16</td>
<td>97±18</td>
<td>388±40</td>
</tr>
<tr>
<td>60</td>
<td>8</td>
<td>15±12</td>
<td>102±19</td>
<td>405±50</td>
</tr>
</tbody>
</table>

Values are Mean±SD, SAP: Systolic arterial pressure, DAP: Diastolic arterial pressure, HR: Heart rate

**Table 2:** Haemodynamic data at the onset of arrhythmias in the antagonist experiments

<table>
<thead>
<tr>
<th>Dose of moxonidine (μg kg⁻¹, IV)</th>
<th>n</th>
<th>SAP (mm Hg)</th>
<th>DAP (mm Hg)</th>
<th>HR (beats min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60+Cont</td>
<td>8</td>
<td>15±20</td>
<td>102±19</td>
<td>405±50</td>
</tr>
<tr>
<td>60+Ef</td>
<td>8</td>
<td>14±23</td>
<td>97±18</td>
<td>41±41</td>
</tr>
<tr>
<td>60+Rau</td>
<td>8</td>
<td>16±14*</td>
<td>113±10</td>
<td>389±49</td>
</tr>
</tbody>
</table>

Values are Mean±SD, SAP: Systolic arterial pressure, DAP: Diastolic arterial pressure, HR: Heart rate, Cont: saline 10 μL, intracerebral, (IC), Ef: efaroxan 20 μg kg⁻¹, IC, Rau: rauwolscine 40 μg kg⁻¹, IC, respectively. *p<0.05 compared with the moxonidine 60+Cont value

**Fig. 1:** Arrhythmogenic dose and plasma concentration of adrenaline in the presence of intravenous (IV) moxonidine (0, 20, 40, 60 μg kg⁻¹) during halothane anesthesia. The values are expressed as Mean±SD and the number of observations is shown in parentheses. Statistical significance; *p<0.05, compared with the Max 0 μg kg⁻¹ value

**Fig. 2:** Arrhythmogenic dose and plasma concentration of adrenaline in the presence of moxonidine (60 μg kg⁻¹, IV) with pretreatment with intracerebral (IC) vehicle, efaroxan and rauwolscine during halothane anesthesia. The values are expressed as mean ± SD and the number of observations is shown in parentheses. Mox 60: moxonidine 60 μg kg⁻¹, IV, Cont: saline 10 μL, IC, Ef: efaroxan 20 μg kg⁻¹, IC, Rau: rauwolscine 40 μg kg⁻¹, IC. Statistical significance; *p<0.05, compared with the Max 0+Cont value
Table 3: Hemodynamic data at the onset of arrhythmias in the presence of moxonidine (0–60 µg kg⁻¹ IV) in intact and bilaterally vagotomized rats and in the presence of moxonidine (60 µg kg⁻¹ IV) in rats pretreated with intravenous atropine methylate (5 mg kg⁻¹).

<table>
<thead>
<tr>
<th>Dose of Moxonidine (µg kg⁻¹, IV)</th>
<th>n</th>
<th>SAP (mmHg)</th>
<th>DAP (mmHg)</th>
<th>HR (beats min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8</td>
<td>134±18</td>
<td>86±17</td>
<td>416±47</td>
</tr>
<tr>
<td>60</td>
<td>8</td>
<td>156±20</td>
<td>102±19</td>
<td>405±50</td>
</tr>
<tr>
<td>Vagotomized rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8</td>
<td>150±19</td>
<td>93±16</td>
<td>464±85</td>
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<tr>
<td>60</td>
<td>8</td>
<td>137±21</td>
<td>92±24</td>
<td>474±92</td>
</tr>
<tr>
<td>Atropine methylate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>8</td>
<td>130±12</td>
<td>84±9</td>
<td>424±66</td>
</tr>
</tbody>
</table>

Values are mean±SD; SAP: Systolic arterial pressure, DAP: Diastolic arterial pressure, HR: Heart rate.

Fig. 3: Arrhythmogenic dose and plasma concentration of adrenaline in the presence of moxonidine (0–60 µg kg⁻¹, IV) in the intact, bilaterally vagotomized rats and atropine methylate (5 mg kg⁻¹, IV)-treated rats during halothane anesthesia. The values are expressed as Mean±SD and the number of observations is shown in parentheses. Statistical significance; *p<0.05, compared with the Max 0 µg kg⁻¹ value (n = 8).

DISCUSSION

The principal finding of this study was that systemically administered moxonidine exerted antiarrhythmic effects in a halothane-adrenaline-induced animal model of arrhythmias via stimulation of I1 receptors in the central nervous system.

The role of imidazoline receptors in the physiological regulation of the central nervous system is well recognized (Parini et al., 1996; Michel and Ernsberger, 1992). For example, Bousquet et al. (1984) reported that imidazoline receptors, rather than central α1 adrenoceptors, mediate the hypotensive effect of clonidine. Imidazoline receptors can be subdivided into the I1 and I2 subtypes (Parini et al., 1996; Michel and Ernsberger, 1992) and the highly selective I1 receptor agonist, moxonidine, was originally developed as an antihypertensive agent (Vanden Zwieth, 1997). In fact, moxonidine has 100,000-fold greater affinity for the I1 receptor subtype than for the I2 receptor subtype (Wickberg et al., 1991; Lione et al., 1996), making this drug a good tool to investigate the physiologic role of I1 receptors. A previous study reported that intravenous moxonidine at 40 µg kg⁻¹ induced hypotension in rats in vivo (Haish et al., 1994). In addition, Lepran and Papp, 1994 showed that 30–100 µg kg⁻¹ of intravenous moxonidine prevented arrhythmias induced by coronary reperfusion. On the basis of these data, a dose range of moxonidine (0–60 µg kg⁻¹, IV) was selected for investigation in the present study.

Since previous studies have reported that I1 receptors are expressed in cardiac tissue (El-Ayoubi et al., 2002) and that moxonidine modulates norepinephrine release by binding to presynaptic receptors (Schäfer et al., 2002), it is unclear whether the central mechanism is involved in the antiarrhythmic effects of systemically administered moxonidine. The present data showed that the antiarrhythmic effect of systemically administered moxonidine was inhibited by central administration of efaxoxan, suggesting that antiarrhythmic effect of systemically administered moxonidine is mediated by I1 receptors in the central nervous system. This is also supported by observations that the effect of moxonidine was completely abolished by bilateral vagotomy (Fig. 3) and by atropine methylate, which suggests that the vagal efferent activity is a critical component of this phenomenon.

It should be noted that moxonidine and efaxoxan also have weak affinity for α2 adrenoceptors (Ernsberger et al., 1992). However, rauwolscine, an α2 adrenoceptor antagonist with little affinity for imidazoline receptors, had little effect on the antiarrhythmic action of the highest dose (60 µg kg⁻¹) of moxonidine, while efaxoxan, an α2 adrenoceptor antagonist with high affinity for I1 receptors, completely inhibited the effect of moxonidine (Fig. 2). These data suggest that moxonidine exerts its antiarrhythmic effect through I1 receptors and not through α2 adrenoceptors.

Haemodynamic parameters, including arterial blood pressure and heart rate, are important factors that can influence the onset of halothane-adrenaline-arrhythmias (Reynolds, 1984; Atlee and Bosnjak, 1990). However, in the present study, haemodynamic parameters at the arrhythmias in the moxonidine-treated rats were not significantly different from those of the control rats.
despite larger plasma adrenaline concentrations in the moxonidine groups (Fig. 1, Table 1). These data suggest that moxonidine attenuated the positive isotropic and chronotropic action of adrenaline via activation of the parasympathetic nervous system, thereby preventing arrhythmias.

CONCLUSION

Systemically administered moxonidine prevents the halothane-adrenaline-induced arrhythmias through activation of central, but not peripheral, 1, receptors.

REFERENCES


