Protective Effects of I1-Antihypertensive Agent Moxonidine against Neurogenic Cardiac Arrhythmias in Halothane-Anesthetized Rabbits

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ABSTRACT

Numerous studies have addressed the antihypertensive properties of I1-imidazoline receptor agonists such as moxonidine, but very few authors examined their cardiac antiarrhythmic potency. Due to the important role of the sympathetic nervous system in the genesis of neurogenic cardiac arrhythmias, we investigated the antiarhythmic effects of moxonidine and compared them to those of propranolol in an experimental model of neurogenic arrhythmias. Chronic bipolar electrodes were implanted within the posterior hypothalamus of six halothane-anesthetized rabbits. Every 15 days, after three 10-min-interval control electrical stimulations, we compared the effects of randomized i.v. administrations of moxonidine (25 μg/kg), propranolol (0.5 mg/kg), and saline (0.9% NaCl) on mean arterial pressure (MAP), heart rate (HR), and ECG during 2.5 h with six stimulations every 20 min. We observed that: 1) in control conditions, intrahypothalamic stimulation increased MAP (ΔMAP = 17 ± 2 mm Hg) and HR (ΔHR = 60 ± 1 beats/min), and triggered extrasystoles (number of extrasystoles = 55 ± 2) and abnormal complexes (number of abnormal ECG complexes = 37 ± 1), which occurred with a 6.4 ± 0.4-s delay and 33 ± 1-s duration; 2) moxonidine and propranolol induced almost equihypotensive (ΔMAP = −12 ± 2 and −10 ± 2 mm Hg) and pronounced bradycardic effects (ΔHR = −47 ± 10 and −78 ± 9 beats/min, respectively). Arrhythmias were significantly reduced by moxonidine and propranolol: Δnumber of extrasystoles = −83 and −98%; Δnumber of abnormal ECG complexes = −33 and −79%; Δdelay = +65 and +188%; Δduration = −35 and −58%, respectively. Our results show that moxonidine presents an antiarrhythmic potency comparable to that of propranolol that should be predominantly related to their central action. However, additional studies are required to determine whether these antiarrhythmic effects are of central and/or peripheral origin.

The antihypertensive properties of clonidine (Schmitt, 1977), rilmenidine (Sannajust and Head, 1994), and moxonidine (Ernsberger et al., 1993) have been largely described, but very few authors determined the cardiac antiarrhythmic properties of these compounds. These centrally acting antihypertensive agents were originally considered as preferential agonists of presynaptic α2-adrenoceptors and developed to inhibit the activity of the sympathetic nervous system. In addition, it is well established that the sympathetic nervous system plays an important role in the genesis of certain types of arrhythmias (Manning and de van Cotten, 1962; Hayashi et al., 1991). Therefore, imidazoline compounds, which interact via imidazoline receptors (IRs) and α2-adrenoceptors with the activity of ortho- and parasympathetic nervous systems, may present cardiac interests in therapeutics.

Since the first studies from Lathers et al. (1978) and Helke et al. (1979), it has been clearly confirmed that the central nervous system contributes to the antiarrhythmic side effects of numerous antihypertensive agents such as β-blockers and ganglionic blocking agents, as well as α2-adrenoceptor agonists. Subsequently, Thomas and Tripathi (1986), then Hayashi et al. (1991), showed that α2-adrenoceptors were involved in the central control of neurogenic arrhythmias and that hyperactivation of the adrenergic tone is a triggering factor of several cardiac rhythm disorders (e.g., ventricular, junctional, and atrial arrhythmias). However, their mechanism of action, initially related to stimulation of central α2-adrenoceptors (Schmitt, 1977), was brought under review by various studies (Bousquet et al., 1984; Ernsberger et al., 1990), leading to the concept of the existence of the new class of IRs. It has been suggested that IRs, mainly located in the rostral ventrolateral medulla oblongata, are involved in the central regulation of arterial blood pressure (BP), and that stimulation of these receptors induces a sympathoinhibitory effect with direct impact on the heart, kidneys, and vasculature (Göthert and Molderings, 1992).

ABBREVIATIONS: IR, imidazoline receptor; AC, abnormal ECG complex; NbAC, number of abnormal ECG complexes; BP, blood pressure; ES, extrasystoles; HR, heart rate; IHR, initial heart rate; IMAP, initial mean arterial pressure; MAP, mean arterial pressure; NbES, number of extrasystoles; SHR, maximal heart rate under hypothalamic stimulation; SMAP, maximal mean arterial pressure under hypothalamic stimulation.
In the light of this concept of IR, we planned to examine the antiarrhythmic properties of one of the most selective I$_1$ subtype of IR agonist, moxonidine. Its effects have been previously studied in conscious Sprague-Dawley rats with acute coronary occlusion without reperfusion, or when anesthetized, with reperfusion (Lepran and Papp, 1994). These authors showed that a preventive i.v. treatment with moxonidine significantly reduced both ischemic and reperfusion arrhythmias. Furthermore, a recent study reported potent antiarrhythmic effects of moxonidine on ouabain-induced cardiac arrhythmias in guinea pigs (Mest et al., 1995).

The role of the central and autonomic nervous systems in cardiac diseases has been clearly demonstrated in experimental and clinical studies (Randall et al., 1976; Burch, 1978). Historically, the hypothalamus has been recognized as one of the major structures involved in the cardiovascular responses induced by emotion and stress, and observed during defense reaction in both animals and humans. Electrical stimulation of various diencephalic structures has been shown to induce extrasystoles (ES), ventricular tachycardias, and other types of arrhythmias in rabbits (Evans, 1976; Zhou et al., 1994), cats (Fuster and Weinberg, 1960; Manning and de van Cotten, 1962; Evans and Gillis, 1978), dogs (Verrier et al., 1975), or rats (Mormugo, 1968). From such studies, we have chosen to electrically stimulate the posterior hypothalamus of halothane-anesthetized rabbits to obtain a reproducible experimental model of cardiac neurogenic arrhythmias.

Propranolol, a Vaughan-Williams (1984) class-II antiarrhythmic agent, belongs to the series of $\beta_1$/$\beta_2$-blockers. It exerts a membrane stabilizing effect, by specifically increasing the electrical stability of the myocardium, and is devoid of partial adrenoceptor agonist properties. Moreover, Huang (1969) showed that propranolol (0.1–4 mg/kg i.v.), 5 min postinjection, prevents the development of cardiac arrhythmias triggered by intrahypothalamic electrical stimulation in anesthetized cat, and these effects can persist from $\sim$20 to 30 min. In Wistar rats, Elghozi et al. (1979) demonstrated that after i.v. injection, propranolol diffuses into hypothalamic and medullary structures. The retention by nuclei involved in BP and cardiac rate regulation may be related to the antihypertensive and antiarrhythmic actions via an inhibitory pole and the shaft to the positive pole. Stimulation was monitored and maintained at 38–39°C with a heating pad (Harvard homeothermic blanket control unit; Ealing S.A., Les Ulis, France).

Each rabbit was placed in a standard Horsley-Clarke stereotoxic device (Sociétè Réalisation-Application Mécanique, Sartrouville, France) with a specific rabbit adaptor (Chatelier and Buser, 1961). Then a 5-mm-diameter hole was made with a surgical drill through the skull and a stainless steel, concentric, bipolar electrode (Rhodes Medical SNE 100 with connector, 21 mm length, 0.5 mm o.d.; Phymep S.A., Paris, France) was inserted into the posterior hypothalamus according to the following stereotaxic coordinates related to the interaural line: A14.5 to A15.5; L0.5 to L1; H $+$5 of Urban and Richardson’s atlas (1972) with the corrections due to the adaptor (Poisson et al., 1974). The electrode was connected to a power supply circuit delivering specific electrical stimulations. The implantation zone was determined as a function of the quality and quantity of ECG disorders triggered by preliminary stimulations, and then the electrode was subsequently solidarized to the cranial surface with three post screws (RRA-S1; Dentatus S.A., Hagersten, Sweden) and dental cement (Texton; SS-White, Gloucester, UK). Three s.c. stainless steel ECG electrodes (23 gauge, 19 mm; Ets Polylabo S.A., Strasbourg, France) were introduced under the skin of forearms and hindlimbs of each animal for continuous ECG signals acquisition from standard leads I, II, and III.

**Materials and Methods**

**Animals.** Six male normotensive Zika rabbits, aged 10 to 12 months and weighing 3.2 ± 0.3 kg, were used. They were obtained from the Valteau colony (Valteau S.A., Bressuire, France) and were kept in our laboratory animal house for an acclimatization period of 8 to 12 days before use. The rabbits were maintained under standard conditions of temperature (21 ± 1°C), lighting (12-h light/dark cycle; lights on from 8:00 AM–8:00 PM; 100 ± 20 lux at cage level), humidity (60 ± 10%), and nonrecycled air, changed 15 to 20 times per hour. They received standard diet 112 (UAR Society, Villemeison-sur-Orge, France) containing less than 0.3% sodium, and water ad libitum. The study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals presented by the National Institutes of Health.

**Surgical Preparation.** Under local anesthesia (2% xylocaine; Astra-France Laboratories, Nanterre, France), a Teflon catheter (Jelco 22G, 25 mm; Johnson & Johnson Laboratories, New Brunswick, NJ) was inserted into the ear medial artery, then connected to a pressure transducer (RP 1500; Roucaire, Velizy-Villacoublay, France) for arterial BP measurement. A Teflon catheter (Jelco 24G, 19 mm; Johnson & Johnson Laboratories) was introduced into the ear marginal vein for acute i.v. administration of substances.

After induction of anesthesia with propofol (Diprivan 1%, 10–20 mg/kg i.v.; Zeneca-Pharma Laboratories, Cergy, France) and endotracheal cannulation (pediatric tube, 2.5 mm i.d.; Cole Foregger, New York, NY) each animal was connected to an inhalator (Fluo gente Mark II; Cyprone, Keighley, UK), allowing spontaneous breathing of a mixture (0.2–4%) of halothane (Fluothane; Zeneca-Pharma Laboratories) with room air, throughout the duration of electrode implantation and experimentation. Body temperature was monitored and maintained at 38–39°C with a heating pad (Harvard homeothermic blanket control unit; Ealing S.A., Les Ulis, France).

During each experiment, the electrode of stimulation was connected to a main stimulator (Physiovar TR; Alvar, Bordeaux, France) associated with two high-frequency isolators (Rhodes Medical SNE 100 with connector, 21 mm length, 0.5 mm o.d.; Phymep S.A., Paris, France) and then the electrode was subsequently solidarized to the cranial surface with three post screws (RRA-S1; Dentatus S.A., Hagersten, Sweden) and dental cement (Texton; SS-White, Gloucester, UK). Three s.c. stainless steel ECG electrodes (23 gauge, 19 mm; Ets Polylabo S.A., Strasbourg, France) were introduced under the skin of forearms and hindlimbs of each animal for continuous ECG signals acquisition from standard leads I, II, and III.

**Experimental Protocol.** During each experiment, the electrode of stimulation was connected to a main stimulator (Physiovar TR; Alvar, Bordeaux, France) associated with two high-frequency isolation units. The stimulator delivered two successive 1-ms rectangular pulses from opposite polarity, with amplitude stimulation from 6 to 12 V and frequency from 20 to 120 Hz. These latter parameters were varying in function of delay between surgical implantation and experimental series and degree of anesthesia. This stimulator was programmed by another stimulator (Stimulator-T; Hugo Sachs Elektronik, Hugstetten, Germany) which, by means of a time switch, delivered impulse trains for 30 s every 10, 20, or 45 min. The bipolar electrode was subsequently connected to two outputs of the two high-frequency links in series with the center connected to the negative pole and the shaft to the positive pole. Stimulation was monitored by a scope (THS 720A; Tektronix S.A., Les Ulis, France) mounted in parallel with the circuit, enabling accurate adjustment of voltage stimulation and maintenance of a constant amplitude throughout the duration of experimentation.

Then, the arterial catheter was connected to a direct BP transducer and systolic BP, diastolic BP, heart rate (HR), and the ECG biopotentials were continuously recorded on a physiograph (Desk...
Based on the atlas of Urban and Richard (1972) (see Fig. 1), stimulating sites identified according to the stereotaxic landmarks columna fornicis; mt, tractus mammillotegmentalis; to, tractus opticus.

Area hypothalamica posterior; HVM, nucleus ventromedialis hypothalami; HYL, area hypothalamica lateralis; RET, nucleus reticularis thalami; cfx, columna fornici; mt, tractus mammillotegmentalis; to, tractus opticus.

Fig. 1. Schematic diagrams of coronal brain sections close to anterior planes A14.5, A15, and A15.5 (millimeters) related to the interaural line according to the stereotaxic atlas of Urban and Richard (1972) and demonstrating the distribution of stimulation sites (●) in six rabbits. CD, nucleus caudatus; HD, area hypothalamica dorsalis; DMD, nucleus dorsomedialis hypothalami; HDM, nucleus hippocampus dorsalis; A15, hippocampus ventralis; HP, area hypothalamica posterior; HVM, nucleus ventromedialis hypothalami; HYL, area hypothalamica lateralis; RET, nucleus reticularis thalami; cfx, columna fornici; mt, tractus mammillotegmentalis; to, tractus opticus.

Propranolol HCl was purchased from Sigma Chemical Company (L’Isle d’Abeau, France) and administered i.v. at a dose of 500 μg/kg.

Sterile saline (NaCl 0.9%), moxonidine (25 μg/kg), and propranolol (500 μg/kg) were administered i.v. under a constant 250-μl volume and followed by a 400-μl injection of sterile saline.

Data Analysis. Systolic BP, diastolic BP, HR, and ECG values continuously recorded on the chart physiograph were manually measured off-line. The maximal values of mean arterial pressure (MAP) and HR induced by each hypothalamic electrical stimulation (SMAP and SHR, respectively) were determined at the peak value observed during the 30-s stimulation and after the 2-min periods; they were then compared with MAP and HR initial values (IMAP and IHR, respectively) measured over the minute preceding each stimulation.

Arrhythmias of atrial or ventricular origins presented several kinds of forms: isolated or by bursts of bigeminy, atrial or ventricular tachycardias, torsades de pointes, auriculoventricular blocks, and the QRS and T waves alterations (Fig. 2). They were quantified by: 1) the number of extrasystoles (NbES) as premature atrial or ventricular complexes; 2) the number of abnormal ECG complexes (NbAC), consisting of ECG disorders of repolarization or conduction type. Disorders of repolarization were based on reference standard values reported by Kossakowski and Kuczynski (1973) in anesthetized rabbits. In our experimental conditions, the values observed in lead II were: magnitude of T wave: 0.1 ± 0.02 mV, QT interval: 80 ± 6 ms and broad QRS interval: 40 ± 4 ms. Abnormal values observed in magnitude of T wave (range: 0.17–0.3 mV), QT interval (range: 100–160 ms), and broad QRS interval (range: 60–100 ms) were therefore considered as abnormal ECG complexes (ACs). In addition, the deeply inverted T waves, as well as cardiac auriculoventricular blocks have also been quantified as AC; 3) the delay (d) of initiation of arrhythmias after the onset of hypothalamic electrical stimulation and 4) the total duration (D) of arrhythmias for each period of stimulation.

The hypotensive and bradycardic effects of moxonidine and propranolol were measured during the first 15 min after injection and...
Fig. 2. Representative traces showing the effects of hypothalamic stimulation on arterial blood pressure (ABP) and ECG leads II and III in one halothane-anesthetized rabbit in: control condition, during the first stimulation (66 Hz, 7 V) (A); during the fourth stimulation, 15 min after moxonidine (25 μg/kg i.v.) and 15 days later in the same rabbit (B); during the third control stimulation (100 Hz, 10 V) (C); and during the fourth stimulation, 15 min after propranolol (500 μg/kg i.v.) (D). Time scale indicates the delay (seconds) after the onset of stimulation. A, typical ventricular ES and two bursts of ventricular tachycardia [global parameters of arrhythmias were 117 ES, 46 AC, onset delay of arrhythmia (d) = 8 s, duration of arrhythmia (D) = 36 s] associated to a fall in ABP after an initial hypertension. B, antiarrhythmic action of moxonidine characterized by a suppression of ventricular tachycardia, decrease in NbES (22), NbAC (45), and arrhythmias duration (d = 12 s, D = 30 s). C, similar succession of various ectopic beats (86 ES, 42 AC, d = 7 s, D = 39 s) to (A). D, pronounced inhibition of arrhythmias after propranolol (0 ES, 2 AC, d = 16 s, D = 2 s).
successively during the minute preceding each intrahypothalamic stimulation (S4, S5, S6, S7, S8, and S9).

Results are presented as means ± S.E. (n = 6 rabbits). For all parameters the effects of moxonidine were compared with those of propranolol- and saline-injected groups, using a two-way ANOVA for repeated measures. The significant differences between MAP and HR values measured before and during stimulation were determined for each treated group using Student’s t test. In addition, for every postinjection stimulation, we compared the effects of each treatment to the values measured before injection and to the effects of other drugs, pairwise, by using the contrast method (SYSTAT software; Evanston, IL), and P values less than .05 were considered significant.

Results

Anatomical Verification of Electrode Implantation Sites. The accuracy of intrahypothalamic stimulation sites was checked by measuring the intensity and amplitude of the stimulation-induced arrhythmic responses and post-mortem histological verifications. Brain sections of six rabbits showed three stimulation sites (see Fig. 1) mainly located within the posterior (A14.5, L0.5, H +2 to H +3), dorsal (A15, L0.5 to L1, H +1 to H +3) hypothalamic areas, and the dorsomedial hypothalamic (A15.5, L0.5 to L0.8, H +1 to H +2) nucleus, according to the atlas of Urban and Richard (1972).

Initial Cardiovascular Parameters. In six halothane-anesthetized rabbits, basal average MAP and HR values determined during the minute preceding the three control stimulations (S1, S2, S3) were 61 ± 1 mm Hg and 305 ± 4 beats/min, respectively (n = 54). There was no significant difference in IMAP and IHR values between the three (moxonidine-, propranolol-, and saline-) treated groups of animals.

Control intrahypothalamic stimulations (n = 54) induced a significant (P < .001) increase (28%) in MAP evaluated by the difference measured between SMAP and IMAP of +17 ± 2 mm Hg. The stimulation produced a significant (P < .001) tachycardic (20%) effect determined by the difference between SHR and IHR (ΔHR = +60 ± 1 beats/min) (Fig. 2). The mean values for control group are presented in Table 1. No significant difference during control hypothalamic stimulations was observed between the three groups of measured values (n = 18).

Cardiovascular Effects of Substances. The maximal hypertensive and bradycardic effects of each substance were determined during the 15 min after i.v. bolus injection. Moxonidine (25 µg/kg) significantly (P < .01) lowered MAP: 49 ± 5 versus 61 ± 4 mm Hg (ΔMAP = −12 ± 2 mm Hg) and was almost equihypotensive: 46 ± 4 versus 56 ± 4 mm Hg (ΔMAP = −10 ± 2 mm Hg) to propranolol (500 µg/kg). In contrast, propranolol produced a significant (P < .01) decrease (25%) of resting HR: 234 ± 9 versus 312 ± 9 bpm (ΔHR = −78 ± 9 bpm). This effect was more pronounced after propranolol than moxonidine treatment (16%): 253 ± 8 versus 300 ± 15 beats/min (ΔHR = −47 ± 10 beats/min) (Fig. 3).

Data in Fig. 4 indicate that 1 min before each stimulation (S4, S5, S6, S7, S8, and S9) after injection, each treatment significantly (P < .05) lowered MAP in comparison with the mean IMAP recorded before each control stimulations (S1, S2, and S3). Moxonidine exerted an hypotensive effect: 52 ± 5 versus 62 ± 3 mm Hg (ΔIMAP = −15%), which became significant (P < .05) only 2 h after injection whereas those of propranolol: 47 ± 5 versus 56 ± 3 mm Hg (ΔIMAP = −16%) appeared significant (P < .01) from 55 to 75 min after the injection. The hypotensive action was short-lived (20 min) for propranolol as well as for moxonidine. Propranolol induced a significant (P < .01) decrease in IHR: 219 ± 10 versus 309 ± 11 beats/min (ΔIHRI = −29%) compared with the mean IHR observed before all of the control stimulations (S1, S2, S3) and to the saline-treated group: 300 ± 10 versus 307 ± 10 beats/min (ΔIHR = −2%) and moxonidine treated-group: 280 ± 21 versus 299 ± 17 beats/min (ΔIHR = −6%). This decrease appeared 15 min postinjection and persisted beyond the 140 min of recording.

The stimulation-induced hypertensive effect was significantly (P < .01) lowered by moxonidine: SMAP = 67 ± 4 versus 75 ± 3 mm Hg (ΔSMAP = −11%) from 35 to 55 min postinjection. Propranolol similarly prevented the SMAP increase: 69 ± 3 versus 77 ± 6 mm Hg (ΔSMAP = −10%) but this significant (P < .05) effect only appeared 35 min postinjection. Moxonidine significantly (P < .05) decreased SHR: 324 ± 9 versus 388 ± 28 beats/min (ΔSHR = −16%) 15 min after injection; this effect lasted 60 min. Fifteen minutes after injection, propranolol induced a significant (P < .001) decrease in SHR: 230 ± 8 versus 351 ± 15 beats/min (ΔSHR = −34%), which persisted throughout the duration of recording.

Effects of Control Intrahypothalamic Stimulations on ECG Parameters. The control intrahypothalamic stimulations (S1, S2, and S3) induced for all animals cardiac arrhythmias that were mainly characterized by sinusal or

<table>
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<th>TABLE 1</th>
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<td>Effects of nine intrahypothalamic stimulations on cardiovascular and arrhythmias parameters in six saline-treated anesthetized rabbits</td>
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Data are means ± S.E.

<table>
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<tr>
<th>Stimulation</th>
<th>Time</th>
<th>ΔMAP (mm Hg)</th>
<th>ΔHR (beats/min)</th>
<th>NbES</th>
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<tr>
<td>S1</td>
<td>0</td>
<td>18 ± 6**</td>
<td>38 ± 10**</td>
<td>51 ± 9</td>
<td>39 ± 4</td>
<td>7.7 ± 1.3</td>
<td>32.3 ± 5.2</td>
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<td>S2</td>
<td>10</td>
<td>19 ± 6**</td>
<td>48 ± 16*</td>
<td>52 ± 10</td>
<td>37 ± 4</td>
<td>6.3 ± 0.7</td>
<td>28.4 ± 6.4</td>
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<td>S3</td>
<td>20</td>
<td>19 ± 4**</td>
<td>58 ± 17</td>
<td>53 ± 10</td>
<td>37 ± 6</td>
<td>6.3 ± 0.7</td>
<td>27.5 ± 7.1</td>
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<td>S4</td>
<td>45</td>
<td>20 ± 7**</td>
<td>64 ± 22</td>
<td>51 ± 11</td>
<td>43 ± 7</td>
<td>7.5 ± 1.4</td>
<td>30.7 ± 4.3</td>
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<tr>
<td>S5</td>
<td>65</td>
<td>20 ± 5**</td>
<td>56 ± 16**</td>
<td>45 ± 8</td>
<td>37 ± 5</td>
<td>6.4 ± 0.8</td>
<td>33.6 ± 4.0</td>
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<td>S6</td>
<td>85</td>
<td>19 ± 6**</td>
<td>52 ± 9</td>
<td>45 ± 11</td>
<td>34 ± 5</td>
<td>6.3 ± 0.7</td>
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<tr>
<td>S7</td>
<td>105</td>
<td>19 ± 7**</td>
<td>48 ± 10**</td>
<td>49 ± 8</td>
<td>36 ± 7</td>
<td>7.4 ± 1.2</td>
<td>33.8 ± 3.9</td>
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<td>S8</td>
<td>150</td>
<td>20 ± 5**</td>
<td>56 ± 14**</td>
<td>58 ± 9</td>
<td>32 ± 4</td>
<td>5.4 ± 0.7</td>
<td>36.3 ± 3.0</td>
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<tr>
<td>S9</td>
<td>170</td>
<td>19 ± 6*</td>
<td>40 ± 12</td>
<td>50 ± 7</td>
<td>31 ± 2</td>
<td>6.2 ± 0.7</td>
<td>32.8 ± 2.9</td>
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---, saline injection 30 min after the first stimulation; ΔMAP, difference between SMAP and IMAP; ΔHR, difference between SHR and IHR; d, delay of arrhythmias onset after the start of each stimulation; D, duration of arrhythmias for each stimulation.

Significance of the differences: * P < .05; ** P < .01 (Student’s t test for paired variables).
ventricular ES for whom mean number (NbES) was 55 ± 2 (see Fig. 2 and Table 1 for control group). There was no significant difference in NbES between each treated (saline, moxonidine, propranolol) group of measured values (n = 18). Stimulations induced the emergence of NbAC characterized by broadened QRS intervals, increased T wave, QT lengthening with sometimes torsades de pointes, ST segment depression or elevation, and auriculoventricular conduction disorders, eventually blocks. The mean NbAC observed in animals was 37 ± 1 (Fig. 5). The average delay of arrhythmia onset after poststimulation initiation observed in animals was 6.4 ± 0.4 s, and the mean duration of arrhythmia measured from the first AC extended to 33.1 ± 1.4 s (Fig. 6). All parameters did not present any significant difference between saline, moxonidine, and propranolol treatments.

**Effects of Administration of i.v. Drugs on Arrhythmias.** Moxonidine (25 μg/kg i.v.) induced a persistent (140 min after injection) and significant (P < .01) decrease in NbAC: 10 ± 4 versus 59 ± 6 (ΔNbES = −83%) 15 min postinjection (see Table 2 and Fig. 5). Comparatively, propranolol (500 μg/kg i.v.) markedly (P < .01) decreased NbES: 2 ± 1 versus 53 ± 5 (ΔNbES = −98%) triggered by stimulation in animals; this effect began 15 min after injection and persisted until the end of the experiment. Moxonidine induced a significant (P < .05) decrease in NbES: 10 ± 4 versus 59 ± 6 (ΔNbES = −83%) 15 min after i.v. injection. Propranolol significantly (P < .01) decreased the NbAC induced by stimulation: 14 ± 5 versus 37 ± 2 (ΔNbAC = −79%) 15 min after injection and this effect lasted throughout the duration of recording. The effects of propranolol were significantly (P < .05) more pronounced (ΔNbES = −15%; ΔNbAC = −46%) than those of moxonidine (Table 2). There were no significant effects on NbES and NbAC in the saline-treated group (Table 1).

Moxonidine treatment significantly (P < .01) prolonged the average delay of onset of arrhythmia emergence as shown in Fig. 6 and Table 2. The maximal mean delay value was 12 ± 1 versus 7 ± 1 s (Δd = +65%). This effect started 15 min postinjection and lasted 60 min. In contrast, propranolol did not significantly increase the delay of arrhythmias: 13 ± 4 versus 6 ± 1 s (Δd = +35%). This effect was then attenuated but became significant (P < .01; ΔΔd = −23%) at the end of the experiment (140 min). Propranolol significantly (P < .01) reduced the duration of arrhythmia triggered by stimulations: 15 ± 5 versus 35 ± 2 s (ΔΔd = −58%) but this effect, appearing 15 min after injection, lasted only for 40 min. The comparison of moxonidine and propranolol antiarrhythmic actions did not show any significant difference (Table 2), and saline injection did not modify the delay and duration of arrhythmias.

**Discussion**

The major finding of this study is that the most selective I1 subtype of IR antihypertensive agent, moxonidine, could not only prevent increases in MAP and HR induced by electrical intrahypothalamic stimulation, but also exert potent antiarrhythmic properties. These cardioprotective effects of moxonidine have been demonstrated by using an halothane-anesthetized rabbit arrhythmia model with posterior intrahypothalamic electrical stimulations. Such conditions produced significant hypertensive and tachycardic effects associated with neurogenic cardiac arrhythmias predominantly characterized by premature and ectopic beats, auriculoventricular blocks, and repolarization disorders.

First, we found that an i.v. dose of 25 μg/kg of moxonidine induced, during the 15 min after the injection, significant but moderate decreases in MAP and HR, which were similar to those observed after administration of an i.v. dose of 500 μg/kg of propranolol (class II β-blocking agent of reference). Thus, if moxonidine and propranolol were still equihypotensive 1 h after injection, propranolol revealed to be more bradycardic (+9%) than moxonidine. Moreover, propranolol, like moxonidine, inhibited the intrahypothalamic stimulation-induced hypertension, and this effect lasted 1 h. The two substances prevented the stimulation-induced tachycardia but propranolol effect was longer (>2.5 h) than that of moxonidine (~1.5 h).

Second, moxonidine exhibited pronounced antiarrhythmic effects characterized by profound decreases in NbES and NbAC, prolonged delay of onset of arrhythmias, and important diminution of duration of arrhythmias. These effects were slightly lower than those of propranolol, but lasted beyond the end of each experiment (140 min after injection). This study performed under long-lasting halothane anes-
thesia confirmed the cardiodepressive and arrhythmogenic actions of this anesthetic agent as previously reported by Maze and Smith (1983), Tranquilli et al. (1986), and Hayashi et al. (1993) in anesthetized dogs. Halothane lowered basal MAP (60 ± 6 versus 71 ± 4 mm Hg) and accelerated HR levels (305 ± 6 versus 180 ± 10 beats/min) in comparison with conscious rabbits (Sannajust and Head, 1994). In addition, it is well established that halothane (as chloroform) sensitizes the heart to arrhythmogenic effects of adrenaline, and this property is currently used in experimental animal models of arrhythmias (Caillard and Louis, 1980). Because the intrahypothalamic electrical stimulations are responsible for an elevation in sympathetic tone and adrenaline release from adrenal glands (Stoddard-Apter et al., 1983), halothane potentiated the cardiac arrhythmias in our experimental model. Moreover, it has been shown that the sympathetic nervous system and adrenergic neuromediators (e.g., noradrenaline and adrenaline) are directly involved in the BP and HR increases induced by posterior intrahypothalamic stimulation in anesthetized and conscious cats (Singewald and Philippu, 1996). However, the genesis of cardiac arrhythmias of central origin may be related to a parasympathetic nervous system activation as demonstrated by Manning and de van Cotten (1962) in anesthetized cats. Similarly, the marked bradycardia obtained by Zhou et al. (1994) during electrical stimulation of lateral hypothalamus in isoflurane-anesthetized rabbits confirmed the important role of cardiac vagal innervation in the genesis of arrhythmias.

The choice of moxonidine and propranolol doses used was based on preliminary studies performed in our laboratory and by others (Lepran and Papp, 1994) for which we observed that low doses (10–20 µg/kg i.v.) of moxonidine induced slight antiarrhythmic effects, whereas a higher dose (30 µg/kg i.v.) exerts pronounced and long-lasting bradycardic effects. Furthermore, we found that doses higher than 50 µg/kg i.v. could sometimes provoke respiratory or cardiac arrests in some animals. The dose of propranolol (500 µg/kg) was chosen to be equihypotensive with moxonidine.

In our experiments, the stimulation of specific posterior and dorsal hypothalamic areas (as shown in Fig. 1) produced hypertension and tachycardia, which suggest the involvement of the sympathetic nervous system in the genesis of
these neurogenic cardiac arrhythmias. This sympathetic hyperactivity could be antagonized by central stimulation of: 1) $\beta$-adrenoceptors, as demonstrated by the effects of propranolol in the anesthetized cat with hypothalamic electrical stimulation (Huang, 1969); 2) $\alpha_2$-adrenoceptors, which are stimulated by clonidine in the ouabain-induced guinea pig arrhythmia model (Thomas and Tripathi, 1986); and 3) $I_1$ subtype of IR predominantly stimulated by moxonidine in the same model (Mest et al., 1995). In addition, the hypotensive action of these compounds represents an additional benefit in the prevention of the stimulation-induced BP increases that involve cardiac baroreflex mechanisms responsible for arrhythmias (Evans and Gillis, 1978).

However, if the hypotensive effect of moxonidine in the conscious (Head and Burke, 1991) and anesthetized rabbit has been initially attributed to the stimulation of $\alpha_2$-adrenoceptors (Armah et al., 1988), additional studies demonstrated that the sympathoinhibitory action of moxonidine (Ernsberger et al., 1993; Chan et al., 1996) is more related to stimulation of $I_1$ subtype of IRs mainly located within the rostral ventrolateral medulla oblongata (Haxhiu et al., 1994). Moreover, Chan et al. (1996) showed that the i.v. moxonidine-induced hypotension was antagonized with efaroxan (a mixed $I_1$-IRs and $\alpha_2$-adrenoceptor antagonist) given intracerebrally but not with 2-methoxyidazoxan (one of the most selective $\alpha_2$-adrenoceptor antagonists). Therefore, these findings suggest that the antihypertensive action of moxonidine is preferentially mediated via stimulation of central $I_1$ subtype of IRs.

The antiarrhythmic effects of moxonidine observed in our study confirm those previously reported by Lepran and Papp (1994). These authors observed pronounced antiarrhythmic properties of moxonidine when administered i.v. (10–100 $\mu$g/kg) during the acute phase of experimental myocardial infarction to conscious coronary artery-ligated rats and, when anesthetized, subjected to reperfusion arrhythmias. In addition, Mest et al. (1995) demonstrated that moxonidine (100–400 $\mu$g/kg i.v.) dose dependently increased the threshold for ouabain-induced arrhythmias in guinea pigs. They have shown that moxonidine was 2-fold more effective than clonidine and as effective as propranolol at a 10-fold higher dose. Our experiments show that moxonidine at a relatively
low dose (25 μg/kg i.v.) and propranolol used at a 20-fold higher dose markedly decreased NbES and NbAC in anesthetized rabbits, and confirm previous data reported by Mest et al. (1995). In addition, these authors observed that the antiarrhythmic action of moxonidine was completely prevented with an i.v. pretreatment with efavoxan and was partially suppressed by a pretreatment with idazoxan (a mixed ligand of I1 and I2 subtypes of IR and an α2-adrenoceptor antagonist). Small doses (10 and 100 nM) of moxonidine and propranolol inhibited the appearance of ES induced by application of acolin in isolated preparations of guinea pig atria. In contrast, a relatively high dose (1 μM) of moxonidine was revealed to be ineffective in this model. Mest et al. (1995) suggested that this biphasic action reflects a peripherally mediated effect (only appearing at low doses) without any arrhythmogenic effect (at high doses). Our experimental model of neurogenic arrhythmias, like ouabain infusion-induced arrhythmia model, is characterized by an elevated of sympathetic tone, which could be antagonized by the central sympathoinhibitory action of moxonidine preferentially mediated via stimulation of the I1 subtype of IR (Schäfer et al., 1995).

Furthermore, the chronic implantation of intrahypothalamic bipolar electrodes in our normotensive rabbits allowed us to establish a within animal statistical design, each animal being its own control. Such an experimental model presents the advantages of performing long-lasting experiments and determining the kinetics of action of drugs (such as moxonidine and propranolol), which present prolonged antiarrhythmic potency (see Table 2). Our results showed that the effects of propranolol peaked 35 min postinjection and persisted for more than 2 h, except for the delay of onset and duration of arrhythmias (1 h only). Maximal antiarrhythmic effects of moxonidine were obtained 15 min after injection and, in some cases, exceeded the duration of the experiments (140 min). We observed that the kinetics of the antiarrhythmic action of moxonidine and propranolol was comparable despite the difference in concentration. Likewise, the direct cardiac and central effects, the propranolol-induced bradycardia, are known to contribute to its antiarrhythmic properties. Thus, moxonidine presents a slight bradycardic action at the dose used but no direct cardiac effect, showing that its antiarrhythmic properties, as well as those of propranolol, are probably of central origin (Mest et al., 1995).

In conclusion, the results of this study demonstrate that moxonidine, one of the most selective central I1 subtype of IR antihypertensive agents, presents a dual therapeutic potency. A low dose (25 μg/kg) of moxonidine inducing moderate hypotension and pronounced bradycardia when administered i.v. to halothane-anesthetized normotensive rabbits, can prevent neurogenic cardiac arrhythmias resulting from repeated posterior hypothalamic electrical stimulations. The pronounced and long-lasting reductions in NbES, NbAC, and duration of arrhythmias obtained after moxonidine were comparable to those observed with propranolol treatment, a reference class II antiarrhythmic agent. However, additional studies are required to determine whether these antiarrhythmic effects are of central and/or peripheral origin.

Table 2

<table>
<thead>
<tr>
<th>% Relative Effect</th>
<th>NbES</th>
<th>NbAC</th>
<th>d</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOX versus before injection</td>
<td>-83**</td>
<td>-33*</td>
<td>+65**</td>
<td>-35*</td>
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<tr>
<td>(15 min) (75 min) (55 min) (15 min)</td>
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<td></td>
<td></td>
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<tr>
<td>MOX versus saline</td>
<td>-81*</td>
<td>-25</td>
<td>+81***</td>
<td>-31*</td>
</tr>
<tr>
<td>(15 min) (15 min) (55 min) (55 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRO versus before injection</td>
<td>-98**</td>
<td>-79**</td>
<td>+18</td>
<td>-58**</td>
</tr>
<tr>
<td>(35 min) (35 min) (55 min) (35 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRO versus saline</td>
<td>-96**</td>
<td>-51*</td>
<td>+155</td>
<td>-55*</td>
</tr>
<tr>
<td>(35 min) (15 min) (55 min) (35 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PRO versus MOX</td>
<td>-92*</td>
<td>-74*</td>
<td>+72</td>
<td>-39</td>
</tr>
<tr>
<td>(140 min) (55 min) (35 min) (35 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*delay of arrhythmias onset after the start of stimulation; D, total duration of arrhythmias for each stimulation.

*p < .05; **P < .01; ***P < .001 (Contrast method comparison).

Acknowledgments

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References


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