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Influence of the Central Benzodiazepinergic System on Peripheral Cardiovascular Regulation

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Diazepam is known to have cardiovascular depressive effects through a combined action on benzodiazepinergic receptor and the GABA receptor-chloride ion channel complex. Moreover, it is known that barbiturates also have some cardiovascular regulatory effects mediated by the central GABAergic system. Therefore, this study was undertaken to delineate the regulatory actions and interactions of these systems by measuring the responses of the cardiovascular system and renal nerve activity to muscimol, diazepam and pentobarbital, administered intracerebroventricularly in rabbits. When muscimol (0.03~0.3 μg/kg), diazepam (10~100 μg/kg) and pentobarbital (1~10 μg/kg) were injected into the lateral ventricle of the rabbit brain, there were similar dose-dependent decreases in blood pressure (BP) and renal nerve activity (RNA). The relative potency of the three drugs in decreasing BP and RNA was muscimol > pentobarbital > diazepam. Muscimol and pentobarbital also decreased the heart rate in a dose-dependent manner; however, diazepam produced a trivial, dose-independent decrease in heart rate. Diazepam (30 μg/kg) augmented the effect of muscimol (0.1 μg/kg) in decreasing blood pressure and renal nerve activity, but pentobarbital (3 μg/kg) did not. Bicuculline (0.5 μg/kg), a GABAergic receptor blocker, significantly attenuated the effect of muscimol in decreasing BP and RNA, either alone or with diazepam, and that of pentobarbital in decreasing BP and RNA, either alone or with muscimol. We inferred that the central benzodiazepinergic and barbiturate systems help regulate peripheral cardiovascular function by modulating the GABAergic system, which adjusts the output of the vasomotor center and hence controls peripheral sympathetic tone. Benzodiazepines more readily modulate the GABAergic system than barbiturates.

Key Words: GABA, Benzodiazepine, Barbiturate, Cardiovascular regulation, Rabbit

INTRODUCTION

The cardiovascular system is regulated by various neurotransmitters and hormones of central nervous system (CNS). It is well known that GABAergic receptors play a central role in regulating peripheral cardiovascular function. Central GABAergic receptor agonists decrease arterial pressure and heart rate (Antonaccio & Snyder, 1981; Williford et al, 1980, 1981; Ito & Sved, 1997). These receptors appear to be located in the ventral surface of the medulla, which is involved in inhibiting sympathetic signals from the CNS to the heart and vasculature (Bousquet et al, 1981; Williford et al, 1981; Minson et al, 1994). In addition, GABAergic receptors are also present on neurons in the nucleus ambiguus. However, when these receptors are stimulated, they inhibit the parasympathetic signals to the heart (DiMicco et al, 1979; Takenaka et al, 1996).

Nevertheless, central GABAergic regulation of peripheral cardiovascular function has not yet been clearly demonstrated, because GABAergic receptors

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are structurally linked with benzodiazepine and picrotoxin-sensitive barbiturate receptors in a GABA-benzodiazepine-barbiturate receptor complex, which affects the chloride-channel.

Jones et al (1979) reported that diazepam, a benzodiazepinergic drug, per se, did not alter the heart rate and mean arterial blood pressure in dogs at doses of up to 2.5 mg/kg. When 30 mg/kg diazepam was administered in conscious rats, however, the heart rate and mean arterial blood pressure decreased, while the adrenaline-induced reflex bradycardia was enhanced (Yang et al, 1987; Chu & Lin, 1996). Infusion of picrotoxin, a central barbiturate receptor antagonist, into the cerebroventricular system produced hypertension and tachycardia by stimulating sympathetic forebrain centers (DiMicco et al, 1977). However, if bicuculline, a GABAergic antagonist, is infused into the lateral ventricle of rat brain, a marked increase in systemic blood pressure results from activation of the peripheral sympathetic nervous and vasopressin systems (Choi, 1994; Zanzinger et al, 1994).

Yamada et al (1983, 1984) reported that peripheral cardiovascular function is regulated by the central GABAergic system through a reflexive pathway involving baroreceptors. On the other hand, McCall (1986) reported that picrotoxin and bicuculline, which are competitive GABAergic antagonists, did not alter norepinephrine-induced baroreflexive sympathoinhibition, indicating that the GABAergic system is not involved in this reflex pathway.

The practical effect of the central GABAergic system on peripheral cardiovascular function is still to be defined. In addition, the roles of the central benzodiazepinergic and barbiturate systems, which are linked with and modulate the function of the GAB Aergic system, need to be further elucidated.

The purpose of this study is to delineate the regulatory actions and interactions of the central GABAergic, benzodiazepinergic, and barbiturate systems on the regulation of peripheral cardiovascular function by measuring their effects on blood pressure (BP), heart rate (HR), and renal nerve activity (RNA) as reflected in the peripheral sympathetic tone.

METHODS

Adult rabbits of both sexes, weighing 1.8~2.4 kg, were anesthetized with 60 mg/kg-chloralose given intravenously. The trachea was cannulated with a plastic tube to protect the airway, and a bilateral vagotomy was performed by cutting the vagal nerve trunk near the trachea. The blood pressure in the right femoral artery was measured with an universal amplifier (Gould, model 13-4615-58) coupled to a Statham P23XL pressure transducer, and a continuous record was kept with a Gould Recorder (model 3400). The diastolic arterial pressure was measured from the tracing and expressed in mean±S.E.M. mmHg. The heart rate was recorded simultaneously with a biotachometer (Gould, model 13-4615-66) linked to the output from the blood pressure monitor, and expressed in beats per minute (mean±S.E.M.).

To measure renal nerve activity, the left kidney was exposed retroperitoneally. A branch of the left renal nerve was carefully isolated from the surrounding connective tissues, cleaned and suspended on bipolar electrodes (WPI, O.D.=0.125 mm, a 95% platinum - 5% iridium alloy) fixed to a micromanipulator (Brinkmann) in a pool of mineral oil. Multiunit renal nerve activity was monitored on an ink recorder and an oscilloscope (Kenwood, CS-4025) simultaneously.

In order to test the validity of the nerve preparation, a reflexive decrease of nerve activity was tested by an i.v. injection of phenylephrine (30 μg/kg). A reduction in nerve activity coinciding with the rise

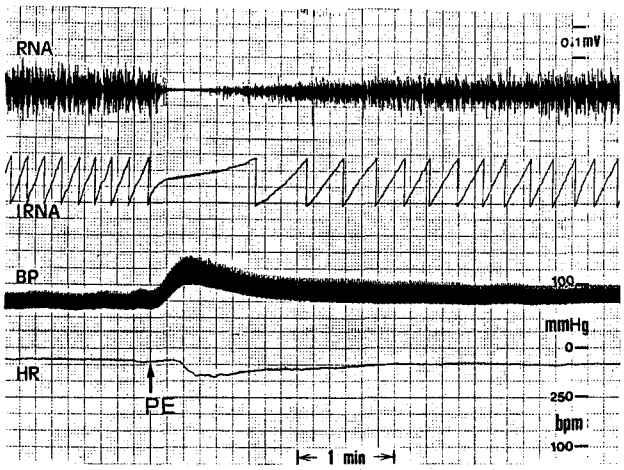

Fig. 1. Effect of phenylephrine (PE) on the renal nerve activity (RNA), blood pressure (BP) and heart rate (HR). PE (30 μg/kg i.v.) was injected to test the validity of the recording system. IRNA means integrated renal nerve activity by 'full scale to reset' integration mode.

in blood pressure confirmed the adquency of the preparation (Fig. 1). Renal nerve activity was quantified with an integrator amplifier (Gould, model 13-4615-70) coupled onto a window discriminator (WPI, model 121) to eliminate noise. All nerve signals above the background noise level were rectified and integrated. The integrator was set in the 'full scale-to-reset' mode, so that the amount of nerve activity was negatively proportional to the reset-time. The change in mean reset-time before and during a test was used for quantitative assessment. The integrated RNA was expressed in a percent change from the control value.

The lateral ventricle of the cerebrum was cannulated for the intracerebroventricular (icv) administration of the agents. A hole was drilled into the skull at a point 1.5 cm from the occipital tubercle and 0.5 cm from the midline, and a polyethylene (PE) cannula (O.D. 1.5 mm) was introduced into the hole obliquely until clear cerebrospinal fluid appeared in the cannula. The cannula was then cemented to the bone. The volume administered did not exceed 0.15 mL. In this report, all agents were given intracerebroventricularly through the cannula unless specified. Drugs administered intravenously (iv) were injected in a volume of 1 mL/kg through an ear vein. To remove the baroreflex-related nerves, the carotid sinus nerves, which are located bilaterally between the external and internal carotid arteries, were isolated and cut, and a bilateral cervical vagotomy was performed.

The drugs used were muscimol HCl (RBI), diazepam (Samjin), pentobarbital sodium (TCI), and bicuculline methiodide (Sigma). Pentobarbital sodium was dissolved in a mixture of 20% propylene glycol, 10% ethyl alcohol, and 70% distilled water. A stock solution of each of the other agents in distilled water was prepared. They were administered after being diluted in the saline solution.

The results were, in some cases, compared using the 'area under the curve (AUTC)', which was the area between the response curve and the base line of the graph (Lesser et al, 1980). The statistical significance of the difference between agents was determined with Student's *t*-test.

RESULTS

Effect of muscimol on blood pressure, heart rate and renal nerve activity

Blood pressure (BP) did not change significantly after icv administration of 0.03 μg/kg muscimol. However, when 0.1 or 0.3 μg/kg muscimol was given, BP fell immediately, decreasing by a maximum of 19±3.8 and 45±5.8 mmHg respectively within 5 min. Afterwards, BP recovered slowly, but remained lower (11±2.9 and 32±8.9 mmHg decreases respectively) for over 30 min as shown in

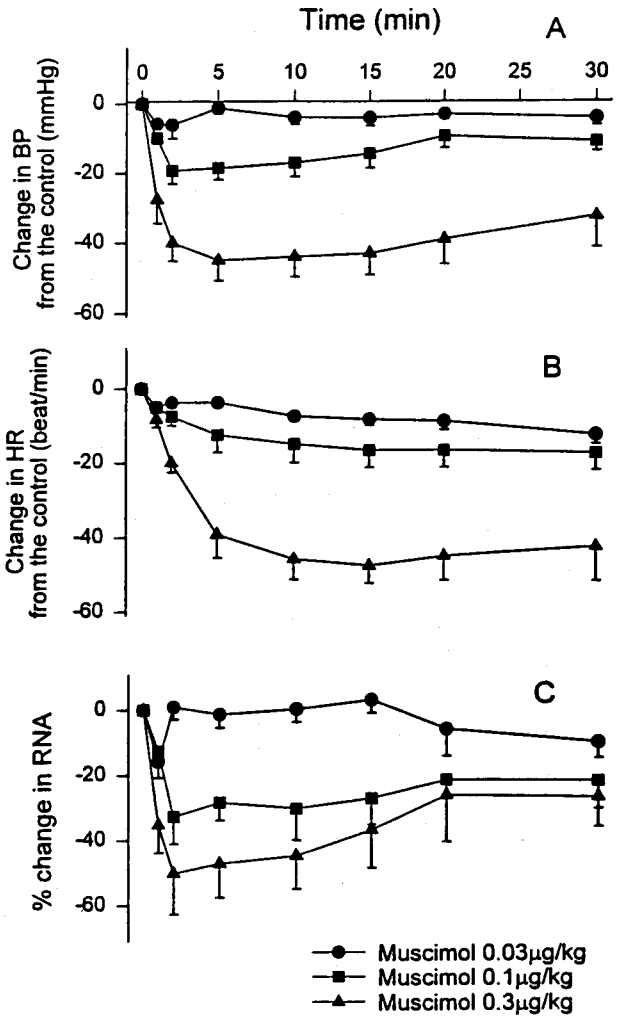

Fig. 2. Effects of muscimol on blood pressure (BP), A, heart rate (HR), B, and renal nerve activity (RNA), C. Muscimol was administered intracerebroventricularly in the volume of 0.15 ml respectively. Each point represents mean±S.E.M. from 5 experiments.

Fig. 2-A.

All three doses of icv muscimol (0.03, 0.1, 0.3 μg/kg) produced an immediate decrease in the heart rate (HR), with maximum respective decreases of 13±2.5, 18±4.6, and 48±4.8 beats/min within 15 min after the administration. This bradycardiac response to muscimol was sustained for over 30 min. The HR decreased in a dose-related fashion (Fig. 2-B).

The administration of 0.03 μg/kg muscimol did not produce any significant changes in renal nerve activity (RNA), but when 0.1 and 0.3 μg/kg of muscimol were given, RNA decreased rapidly, with maximal 32±8.4 and 50±11.6% decreases within 2~3 min. The RNA slowly recovered with the passage of time, but remained lower for over 30 min (22±7.9 and 27±8.7% decreases respectively, Fig. 2-C).

Effect of diazepam on blood pressure, heart rate and renal nerve activity

When 10, 30, and 100 μg/kg diazepam were administered intracerebroventricularly, BP fell instantaneously with maximal dose-related decreases of 12±0.9, 20±10.5 and 29±4.6 mmHg. Subsequently, BP recovered to control levels within 5 min (Fig. 3-A).

However, unlike the case with muscimol, 10, 30 and 100 μg/kg icv diazepam did not produce a significant change in HR. The maximum decrease was 9±2.0, 8±10.6 and 9±4.4 beats/min respectively (Fig. 3-B).

The change in RNA produced by diazepam was similar to that in BP. After the injection of 10, 30, and 100 μg/kg diazepam intracerebroventricularly, RNA fell by 10±6.7, 16±6.1 and 23±9.1%, respectively, within 1 min and then gradually recovered to control levels within 5 min (Fig. 3-C).

Effect of pentobarbital on blood pressure, heart rate and renal nerve activity

Up to 1 μg/kg, icv pentobarbital had no significant effect on BP. However, administration of 3 or 10 μg/kg pentobarbital intracerebroventricularly markedly decreased BP in a dose-dependent manner with maximum respective decreases of 17±3.4 and 38±5.8 mmHg within 5 min. Subsequently, BP recovered slightly but remained 9±2.9 and 16±5.3 mmHg lower for over 30 min (Fig. 4-A).

Similarly, up to 1 μg/kg, icv pentobarbital also had

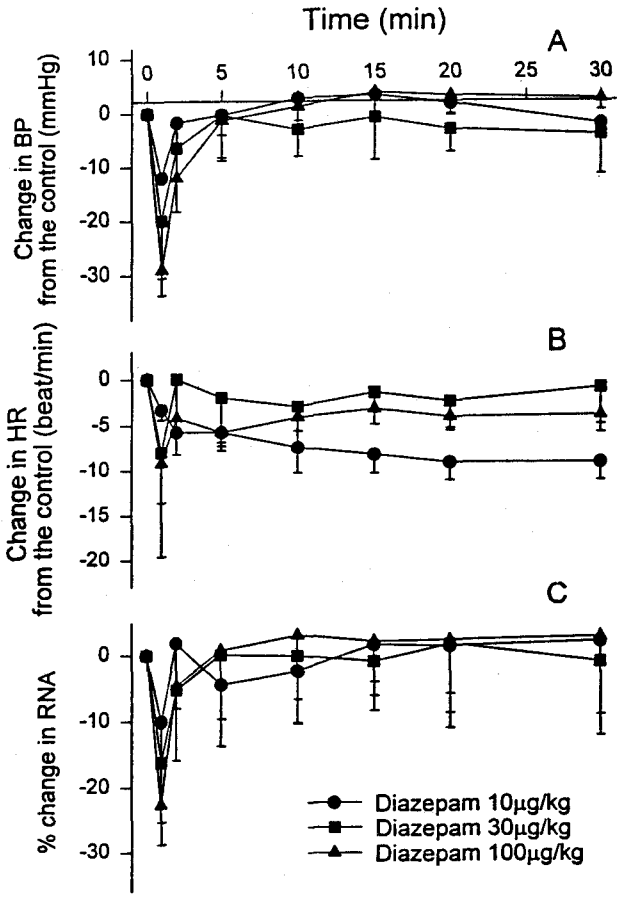

Fig. 3. Effects of diazepam on blood pressure (BP), A, heart rate (HR), B, and renal nerve activity (RNA), C. Diazepam was administered intracerebroventricularly in the volume of 0.15 ml respectively. Each point represents mean±S.E.M. from 5 experiments.

no significant effect on HR, but administration of 3 and 10 μg/kg pentobarbital immediately produced a significant decrease in the HR, with maximum respective decreases of 13±4.8 and 37±6.2 beats/min within 5 min. A bradycardiac response, 9±2.9 and 16±5.4 beats/min lower than normal, was maintained for over 30 min (Fig. 4-B).

Again, 1 μg/kg icv pentobarbital did not produce any significant changes in RNA, but when the amount increased to 3 or 10 μg/kg the RNA reduced maximally by 28±5.5 and 42±10.5%, respectively, within 5 min and in a dose-related manner. There was some recovery, but nerve activity remained reduced by 15±8.5 and 21±9.1% for 30 min (Fig. 4-C).

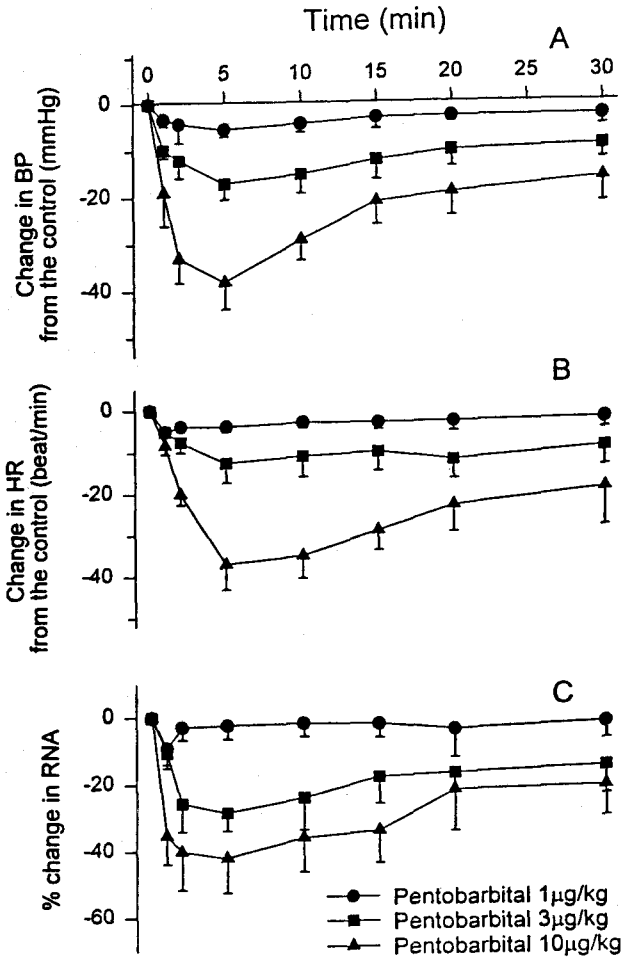

Fig. 4. Effects of pentobarbital on blood pressure (BP), A, heart rate (HR), B, and renal nerve activity (RNA), C. Pentobarbital was administered intracerebroventricularly in the volume of 0.15 ml respectively. Each point represents mean±S.E.M. from 4 experiments.

Effects of diazepam and pentobarbital on blood pressure, heart rate and renal nerve activity in muscimol-treated rabbits

The changes in BP, HR and RNA when the 30 μg/kg diazepam or 3 μg/kg pentobarbital is administered to muscimol (0.1 μg/kg, icv)-treated rabbits were compared with the effects each drug had in untreated rabbits. The area between the base line and the response curve (AUTC) of each test group (a muscimol plus diazepam group, muscimol plus pentobarbital group, and each muscimol-, diazepam- or pentobarbital-alone group) was calculated for the 20 min interval after the administration of the drugs, and the results were compared using Student's *t*-test.

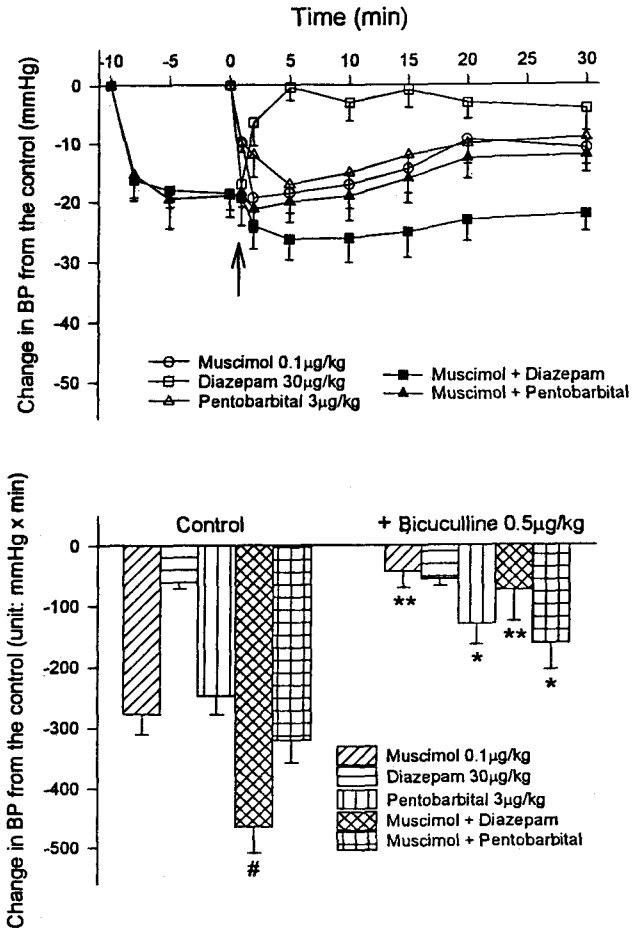

Fig. 5. *Upper*; Time-response curves of intracerebroventricular muscimol, diazepam, pentobarbital, and of muscimol or pentobarbital preceded by muscimol on blood pressure (BP). *Lower*; Effects of the drugs on BP expressed by the area under the curve (AUTC), calculating the area between the base line and the response curves from the upper graphs for 20 min. Left panel shows the effects of respective drugs on BP and right panel shows those of respective drugs after bicuculline pretreatment. Diazepam or pentobarbital was added at ↑. Each point represents mean±S.E.M. from 5 experiments. Each drug was administered intracerebroventricularly. # denotes significant difference from the muscimol alone group at $p<0.01$. * and ** indicate significant difference from the muscimol alone group at $p<0.05$ and $p<0.01$ respectively. Each datum represents mean±S.E.M. from 5 experiments.

With respect to blood pressure, the decrease in the muscimol-treated diazepam group (465±44 mmHg · min) was significantly greater than those seen with muscimol (277±33 mmHg · min) or diazepam (60± 11.1 mmHg · min) alone ($p<0.01$). However, the

decrease in the muscimol-treated pentobarbital group (321±37 mmHg · min) was almost the same as those seen with pentobarbital (246±31 mmHg · min) and muscimol (277±33 mmHg · min) alone (Fig. 5).

With respect to heart rate, the decrease in the muscimol-treated diazepam (293±51 beats) and pentobarbital (215±43 beats) groups was greater than with diazepam (42±12 beats) alone, but almost the same as that with either muscimol (267±45 beats) or pentobarbital (240±35 beats) alone (Fig. 6).

The effect on RNA was similar to that on BP. The decrease in the muscimol-treated diazepam group (732±69% · min) was significantly greater than that with diazepam (17±21% · min) or muscimol (526±57% · min) alone ($p<0.05$). However, the decrease in the muscimol-treated pentobarbital group (423±55% · min) was no different from that of muscimol or pentobarbital (422±49% · min) alone (Fig. 7).

Influence of bicuculline on the changes in blood pressure, heart rate and renal nerve activity elicited by muscimol or diazepam alone, or muscimol-treated diazepam or pentobarbital

0.5 μg/kg icv bicuculline transiently increased BP, HR and RNA within 1~2 min. These effects were maintained for 5 min and disappeared within 10 min.

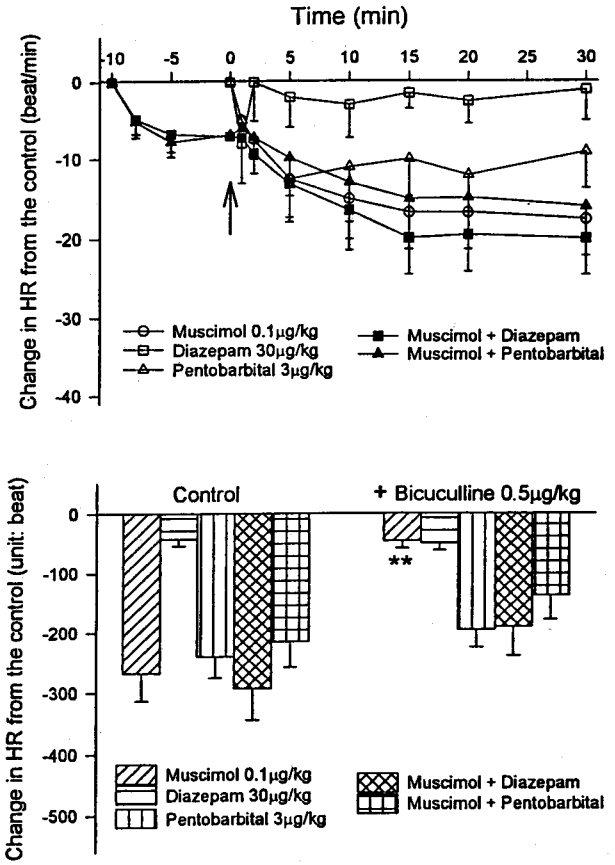

Fig. 6. *Upper*; Time-response curves of intracerebroventricular muscimol, diazepam, pentobarbital, and of muscimol or pentobarbital preceded by muscimol on heart rate (HR). *Lower*; Effects of the drugs on HR expressed by the area under the curve (AUTC). Left panel shows the effects of respective drugs on HR and right panel shows those of respective drugs after bicuculline pretreatment. The other legends are the same as in Fig. 5.

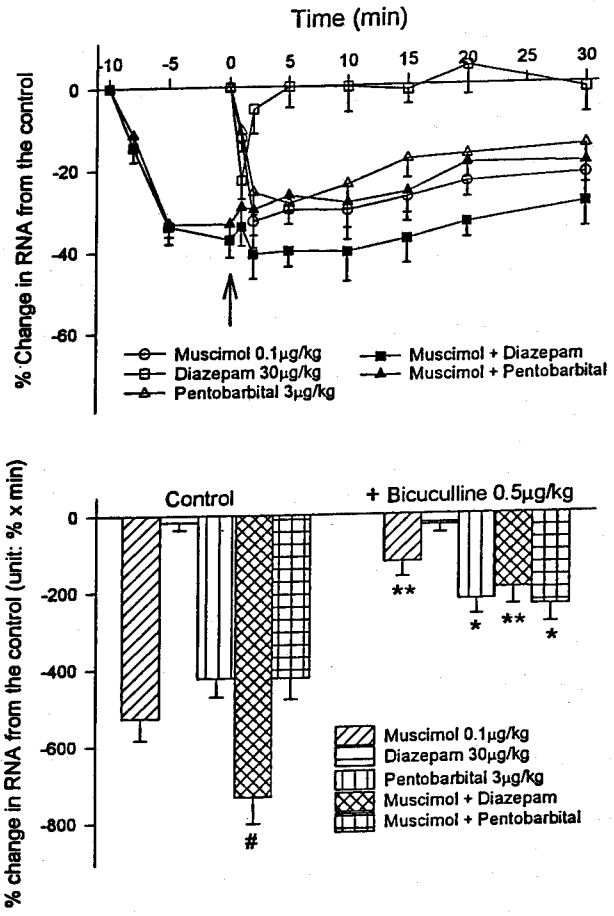

Fig. 7. *Upper*; Time-response curves of intracerebroventricular muscimol, diazepam, pentobarbital, and of muscimol or pentobarbital preceded by muscimol on renal nerve activity (RNA). *Lower*; Effects of the drugs on HR expressed by the area under the curve (AUTC). Left panel shows the effects of respective drugs on RNA and right panel shows the effects of respective drugs after bicuculline pretreatment. The other legends are the same as in Fig. 5.

The effects of muscimol (0.1 μg/kg, icv), diazepam (30 μg/kg, icv), and pentobarbital (3 μg/kg, icv) alone and diazepam and pentobarbital with muscimol on BP, HR, and RNA were measured 15 min after bicuculline administration.

The magnitude of BP decreases was significantly attenuated in all of the bicuculline-treated test groups, except with diazepam alone (60±11.1 mmHg · min). The BP decrease in the muscimol-treated diazepam group compared with muscimol alone was 74±28.0 versus 43±27.3 mmHg · min. Therefore, the synergistic effect of muscimol and diazepam on the fall in BP was not seen in the bicuculline-treated group (Fig. 5).

In the bicuculline-treated groups, the HR decrease was only significantly attenuated by muscimol alone (46±12.0 versus 267±45.5 beats; $p<0.01$, Fig. 6). In the other test groups, there were no significant differences between the bicuculline-treated and non-treated groups.

The magnitude of the decreases in RNA, as in the case of BP, was significantly reduced in all bicuculline-treated groups, except with diazepam alone (29± 21.5% · min, $p<0.01$). With bicuculline pretreatment, the respective magnitudes of the RNA decrease were 195±43.2 and 125±39.1% · min in the muscimol-treated diazepam and muscimol alone groups. These values were not different statistically; in other words, the synergistic effect of muscimol and diazepam on the decrease in RNA disappeared in the bicuculline-treated group (Fig. 7).

DISCUSSION

It is well known that central GABAergic receptors are involved in the regulation of peripheral cardiovascular function (Antonaccio & Snyder, 1981; Gillis et al, 1982; Gebber et al, 1984; Broady et al, 1986; Kubo et al, 1986; Matheson et al, 1986; Choi, 1994; Goren et al, 1997). In addition, the central GABAergic receptor combines structurally with the benzodiazepinergic and barbiturate (picrotoxin) receptors to form a receptor complex, which is connected to the chloride channel. When the GABAergic receptor is activated, the chloride channel opens and there is a chloride ion influx into the cell. This ion-flow evokes hyperpolarization of the cell membrane, and an inhibitory postsynaptic potential (IPSP) ensues (Broady et al, 1994). The central benzodiazepinergic receptor itself does not affect chloride-channel activity. When the GABAergic receptor is stimulated, however, benzodiazepinergic agonists increase the frequency of the chloride channel opening, and barbiturates binding to the picrotoxin-sensitive site prolong the time that the channel remains open. Consequently, activation of this receptor-complex inhibits cellular function by augmenting the IPSP (Twyman et al, 1989; Sigel & Buhr, 1997).

The primary aim of this study was to ascertain the interactive role of the central benzodiazepinergic and GABAergic receptor complex in regulating peripheral cardiovascular function. We measured changes in peripheral cardiovascular function as reflected in blood pressure (BP) and heart rate (HR), and peripheral sympathetic tone as reflected in renal nerve activity (RNA), following intracerebroventricular (icv) administration of GABAergic (muscimol), benzodiazepinergic (diazepam), and barbiturate (pentobarbital) agents.

Although our results showed some differences in the magnitude of the responses, icv muscimol, diazepam, and pentobarbital all decreased BP, HR, and RNA in a dose-dependent manner, which proves that these drugs have an inhibitory effect on the cardiovascular regulation system in the brain. The relative potency of the three drugs in decreasing BP, HR, and RNA was muscimol > pentobarbital > diazepam, although diazepam produced only a transient, non-significant decrease in the cardiovascular parameters and RNA within 5 min of administration at doses up to 100 μg/kg icv. This agrees with a report that benzodiazepine alone does not affect chloride channel activity via the GABA receptor-complex (Twyman et al, 1989). The changing patterns of BP and RNA caused by these three drugs are very similar. The observed changes in BP were caused by a decrease in the sympathetic signals from the vasomotor center in the brain to the heart and peripheral vasculature. The pattern of change in the heart rate differed from those for BP and RNA, suggesting that the change in HR induced by these drugs may be regulated by a different mechanism. McCall (1986) reported that bicuculline and picrotoxin did not affect the norepinephrine-induced baroreflex, indicating that the central GABA receptor-complex does not inhibit autonomic sympathetic nervous activity directly.

The magnitude of the decrease in BP and RNA induced by diazepam combined with muscimol was more severe than that seen by either agent alone,

suggesting that these drugs have a synergistic action on the activation of the chloride channel via the GABA receptor-complex. This supports the hypothesis that diazepam alone does not inhibit cardiovascular function through the GABAergic receptors in the vasomotor center, but inhibits peripheral cardiovascular function via activation of the GABAergic receptors (Gillis et al, 1988; Twyman et al, 1989; Barron et al, 1997).

Bicuculline, a GABAergic receptor blocker, attenuated the synergistic decreases in BP and RNA induced by the combined treatment with muscimol and diazepam, so that the response to the combined agents was no different from that seen with muscimol alone. From this, we infer that the frequency with which the chloride channel opens is not increased by diazepam when the GABAergic stimulation is blocked by bicuculline. Unlike diazepam, pentobarbital and muscimol did not produce synergistic decreases in BP and RNA. Pentobarbital alone decreased BP and RNA more than diazepam alone, and the presence of bicuculline could not completely abolish the response to pentobarbital, indicating that pentobarbital may inhibit the peripheral cardiovascular function through another unknown pathway as well as by activating the chloride channel mediated by the central GABAergic receptor. In fact, it has been reported that barbiturates may inhibit peripheral cardiovascular function by inhibiting the conductivity of the autonomic ganglia (MacDonald & McLean, 1982) and membrane stabilization through a local anesthetic effect (Richter & Holman, 1982) as well as by modulating the GABAergic receptor-complex (Keim & Sigg, 1973; Granger et al, 1995).

In summary, although the results of this study are not sufficient to determine the effects of GABAergic modulation by the benzodiazepinergic or barbiturate systems on the regulation of peripheral cardiovascular function, they do suggest that these systems play a complicated role in decreasing BP and heart rate by way of reducing peripheral sympathetic tone through the modulation of the GABAergic receptor-complex in the vasomotor center and that this is more readily modulated by benzodiazepines than by barbiturates.

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