by inhibiting the Ca sequestering activities of the membranous systems, but it failed to reverse the cooling-induced relaxation of the portal vein. The difference in Ca sequestering activities may be the cause of the differences in the action of sodium vanadate.

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Diazepam facilitates reflex bradycardia in conscious rats

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The effects of diazepam on cardiovascular function were assessed in conscious rats. Intravenous administration of diazepam (1–30 mg kg^{-1}) produced a dose-dependent decrease in both the mean arterial pressure and the heart rate. Also, reflex bradycardia was produced in rats by intravenous infusion of adrenaline (1·25–2·5 μg kg^{-1}). Intravenous pretreatment of the rats with diazepam, although causing no change in the adrenaline-induced pressor effect, did enhance the adrenaline-induced reflex bradycardia. However, the diazepam enhancement of adrenaline-induced reflex bradycardia was antagonized by pretreatment of rats with an intravenous dose of picrotoxin (an agent blocks chloride channels by binding to sites associated with the benzodiazepine-GABA-chloride channel macromolecular complex). The data indicate that diazepam acts through the benzodiazepine-GABA-chloride channel macromolecular complex within the central nervous system to facilitate reflex bradycardia mediated through baroreceptor reflexes in response to an acute increase in arterial pressure.

Benzodiazepines represent a large class of compounds widely used in clinical practice for the treatment of anxiety, insomnia, convulsions and muscular rigidity (Garattini et al 1973; Greenblatt & Shader 1974; Dantzer 1977). Considerable attention has been given to their cardiovascular effects. For example, diazepam, in an intravenous dose of 60 mg, caused a decrease in respiration, blood pressure, left ventricular stroke work and cardiac output (Rao et al 1973). However, to our knowledge, relatively little information is available about the effects of diazepam on reflex bradycardia. The arterial baroreflex system is regarded as one of the most powerful and rapidly acting homeostatic mechanisms for regulating blood pressure (Korner 1971; Kirchheim 1976). Therefore, in the present study, the effects of systemic administration of diazepam or picrotoxin on the reflex bradycardia produced in response to elevation of arterial pressure induced by an intravenous infusion of adrenaline were investigated in conscious rats.

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Materials and methods

Male Sprague-Dawley rats, 250–300 g, were housed individually in wire mesh cages in a room maintained at 22 ± 1·0 °C. They were given free access to tap water and granular chicken feed supplied by Taiwan Sugar Corporation. The animals were anaesthetized with ether. The femoral artery and the femoral vein were catheterized, for blood pressure measurement and intravenous infusion, respectively. Diazepam (1–30 mg kg^{-1}), picrotoxin (0·1–0·3 mg kg^{-1}) or 0·9% NaCl (saline) was administered into the femoral vein. Either the drug or vehicle was administered at a volume of 1·5 mL kg^{-1}. The reflex bradycardia was induced by intravenous infusion of adrenaline (epinephrine, USP, Retired Servicemen's Pharmaceutical Plant of Taiwan) in conscious animals (Lin & Chern 1979; Lin et al 1980). The blood pressure, recorded from the femoral artery, was monitored with a Statham P23Ac transducer, and the heart rate was monitored with a Grass 7C tachometer, triggered by arterial pulses. All recordings were made on a four-channel Grass 7C polygraph.

Results and discussion

Fig. 1 shows that an intravenous dose of diazepam (1–30 mg kg^{-1}) caused a dose-dependent fall in both the heart rate and the mean arterial pressure in conscious animals. For example, after an intravenous dose of 10 mg kg^{-1} of diazepam, both the heart rate and the mean arterial pressure fell almost immediately, and reached their minimum levels (e.g. 20 beats min^{-1} and 12 mmHg, respectively) at 15 min. The cardiovascular responses partially recovered 120 min after the injection of diazepam. The control injection of the saline vehicle caused an insignificant effect on the cardiovascular responses. Table 1 summarizes the vasopressor and bradycardiac responses to intravenous injections of adrenaline in conscious animals. Over the dose range of adrenaline used (1·25–2·25 μg kg^{-1} i.v.), a dose-dependent response was obtained (Lin & Chern 1979). As shown in Table 1, in an animal pretreated with an intravenous dose of 1 mg kg^{-1} of diazepam, the bradycardiac response was significantly enhanced, although the response of the arterial pressure was not significantly different from control. The pretreated animals were given the injection 15 min after the injection of the drug.

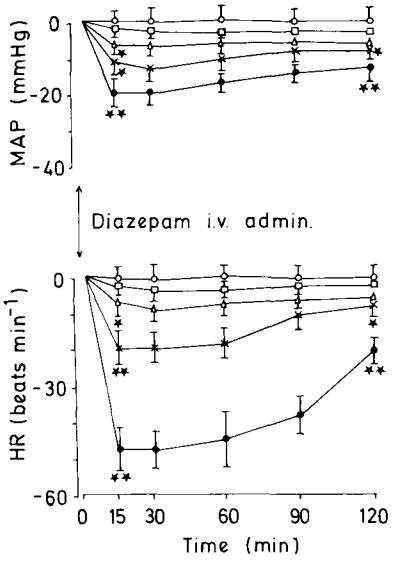

FIG. 1. Dose-response curve for diazepam injected into the femoral vein composed of single injection at each dose level in conscious rats. The points represent the mean falls in heart rate (HR) and mean arterial pressure (MAP) and the vertical bars denote ± s.e. of the means. * Significantly different from the control value (saline group), $P < 0.05$ (Duncan test). ** Significantly different from the control value (saline group), $P < 0.01$ (Duncan test). ○, 0·9% saline (N = 6); □, diazepam 1 mg kg^{-1} (N = 6); △, diazepam 3 mg kg^{-1} (N = 6); ×, diazepam 10 mg kg^{-1} (N = 6); ●, diazepam 30 mg kg^{-1} (N = 6).

On the other hand, intravenous administration of picrotoxin (0·1–0·3 mg kg^{-1}) had no effect on the cardiovascular responses in conscious animals (Table 2). In addition, neither the adrenaline-induced pressor effects nor the adrenaline-induced bradycardia was affected by pretreatment of the animals with an intravenous dose of picrotoxin (Table 1). However, the enhanced bradycardiac responses exerted by diazepam were antagonized by pretreatment of the animals with an intravenous dose of picrotoxin in animals (Table 1). The pretreated animals were given the injection of picrotoxin 10 min before the injection of diazepam or 25 min before the injection of adrenaline.

It is generally believed that the cardiovascular system is regulated by the reciprocal relationships between

Table 1. Effects of administration of diazepam and picrotoxin on the cardiovascular responses induced by intravenous injection of adrenaline in conscious rats.

Treatments	Mean arterial pressure, mmHg			Heart rate, beats min^{-1}		
	Control	After adrenaline	Difference	Control	After adrenaline	Difference
1. Adrenaline 1·25 μg kg^{-1} (i.v.)						
(1) Saline (n = 8)	101 ± 15	142 ± 18	41 ± 4	404 ± 17	350 ± 13	−54 ± 3
(2) Diazepam 1 mg kg^{-1} (n = 8)	103 ± 16	144 ± 18	41 ± 6	403 ± 16	288 ± 11	−115 ± 9*
(3) Picrotoxin 0·2 mg kg^{-1} (n = 5)	99 ± 14	138 ± 16	39 ± 3	400 ± 15	348 ± 12	−52 ± 5
(4) Picrotoxin 0·2 mg kg^{-1} + diazepam 1 mg kg^{-1} (n = 7)	102 ± 17	144 ± 17	40 ± 5	401 ± 18	344 ± 14	−57 ± 5**
2. Adrenaline 2·5 μg kg^{-1} (i.v.)						
(1) Saline (n = 7)	103 ± 14	171 ± 19	68 ± 6	404 ± 15	319 ± 10	−85 ± 6
(2) Diazepam 1 mg kg^{-1} (n = 8)	105 ± 13	170 ± 16	65 ± 5	405 ± 15	255 ± 11	−150 ± 9*
(3) Picrotoxin 0·2 mg kg^{-1} (n = 6)	102 ± 15	170 ± 18	68 ± 7	402 ± 16	322 ± 12	−80 ± 10
(4) Picrotoxin 0·2 mg kg^{-1} + diazepam 1 mg kg^{-1} (n = 8)	101 ± 16	173 ± 19	72 ± 8	403 ± 15	310 ± 14	−93 ± 7**

The values are expressed as mean ± s.e.m.; n = number of rats tested. Adrenaline was administered intravenously 20 min after intravenous administration of diazepam or picrotoxin.

* Significantly different from corresponding control values (saline group), $P < 0.01$ (Duncan test).

** Significantly different from corresponding control values (diazepam group), $P < 0.01$ (Duncan test).

Table 2. Effects of intravenous administration of picrotoxin on the cardiovascular responses in conscious rats.

Treatments[a]	Mean arterial pressure, mmHg			Heart rate, beats min^{-1}		
	Control	Postdrug	Difference	Control	Postdrug	Difference
1. Saline (n = 8)	99 ± 16	103 ± 17	4 ± 2*	401 ± 19	395 ± 19	− 6 ± 3*
2. Picrotoxin 0·1 mg kg^{-1} (n = 8)	101 ± 18	96 ± 14	−5 ± 3*	403 ± 21	399 ± 18	− 4 ± 2*
3. Picrotoxin 0·2 mg kg^{-1} (n = 8)	104 ± 20	102 ± 17	−2 ± 2*	398 ± 18	402 ± 17	4 ± 2*
4. Picrotoxin 0·3 mg kg^{-1} (n = 7)	100 ± 16	103 ± 19	3 ± 2*	402 ± 17	407 ± 20	5 ± 3*

[a] The drug or vehicle solution was administered intravenously at a volume of 1·5 mL kg^{-1}. The values are expressed as mean ± s.e.m.; n = number of rats tested.

* Not significantly different from corresponding control values (saline group), $P > 0·05$ (Duncan test).

vagal tone and sympathetic efferent activity. An increase in arterial pressure stretches the baroreceptors located in the carotid sinus, in the arch of the aorta, and in other large central arteries. Signals from these are transmitted to the brain stem and thence back to the autonomic nervous system to reduce the sympathetic discharge and to increase the vagal discharge; both these effects slow the heart rate and dilate the peripheral blood vessels and restore the blood pressure to normal range (Korner 1971; Kirchheim 1976). The central baroreceptor arc is polysynaptic, with the first synapse located in the nucleus tractus solitarii and the cardiovascular centres (Korner 1971; Kirchheim 1976). In the present results, the reflex bradycardia was induced by intravenous infusion of adrenaline in conscious animals. Pretreatment of the animals with an intravenous dose of diazepam, although causing no distinct change in the adrenaline-induced pressor responses, did potentiate the adrenaline-induced reflex bradycardia. This potentiation was antagonized by pretreatment of the animals with an intravenous dose of picrotoxin. Although picrotoxin is not a direct GABA-receptor antagonist, it blocks chloride channels by binding to sites associated with the benzodiazepine-GABA-chloride channel macromolecular complex. It has also been shown that intracerebroventricular or intracerebral injection of GABA cause hypotension, bradycardia and the enhanced adrenaline-induced bradycardia in rats (Guertzenstein 1973; Willette et al 1983; Yang & Lin 1983). These observations suggest that diazepam acts through the benzodiazepine-GABA-chloride channel complex within the central nervous system to facilitate reflex bradycardia mediated through baroreceptor reflexes in response to an acute increase in arterial pressure. The hypothesis is supported by the findings that the primary action of the benzodiazepines occur at the GABA-ergic synapse where GABA-ergic transmission is facilitated in the presence of the benzodiazepine (Pepeu et al 1980).

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