CENTRAL AND PERIPHERAL CARDIOVASCULAR RESPONSES OF RATS TO GUANABENZ AND CLONIDINE

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Guanabenz (2, 6-dichlorobenzylidene aminoguanidine acetate) is an anti-hypertensive agent (1) possessing a combined structure of the active moieties of both clonidine and guanethidine. The primary mechanism of the antihypertensive action is thought to be mainly mediated via a clonidine-like agonistic action on central \( \alpha \)-adrenoceptors (2). Guanabenz also inhibits peripheral adrenergic transmission via presynaptic \( \alpha \)-receptor stimulation, and there was no evidence showing a guanethidine-like adrenergic neuron blocking activity (2). However, in a previous paper (3), we first differentiated the dual inhibitory actions of guanabenz on peripheral adrenergic transmission, presynaptic \( \alpha \)-receptor stimulation and adrenergic neuron blockade. In the present experiments, we attempted to clarify whether or not these peripheral actions of a moderate concentration of guanabenz play a role in the cardiovascular responses of rats, in comparison with clonidine and guanethidine. We compared the cardiovascular responses of anesthetized rats with those of pithed rats, the spinal cord of which was continuously stimulated at a low frequency of 2 Hz. This frequency was selected so that the basal heart rate and blood pressure was matched to that in anesthetized rats as much as possible.

Male normotensive Wistar rats, weighing 250 to 300 g, were anesthetized with ether, and the left femoral artery and vein were cannulated. The trachea was cannulated, and the animal was pithed through one orbit to the sacral region with a steel rod 1.0 mm in diameter as described by Gillespie and Muir (4) and immediately artificially respired. Atropine at 1 mg/kg and \( d \)-tubocurarine at 1 mg/kg was intravenously injected through the cannula. The spinal cord was continuously stimulated electrically by 1 msec supra-maximal (50 V) pulses at a frequency of 2 Hz by use of an electronic stimulator (Model DPS-10, Dia Medical System). The pithing rod served as the stimulating electrode and a steel needle with an outer diameter of 0.5 mm placed subcutaneously in the dorsum as an indifferent electrode. Some rats were anesthetized with sodium pentobarbitone, 50 mg/kg injected i.p., and the left femoral artery and vein were cannulated.

Arterial blood pressure recordings were made via the femoral cannula using a Toyo pressure transducer (Model MPU-0.5–290) connected to a Sanei recorder (Biophysigraph 180 System). Mean blood pressure was calculated as diastolic pressure +1/3 (systolic pressure - diastolic pressure). Heart rate was continuously computed from the blood pressure pulse wave by a cardiotachometer (Sanei-2140). Drugs used were guanabenz (Nipponshoji), clonidine hydrochloride (Boehringer), guanethidine hemisulfate (CIBA-Geigy), atropine sulfate, and \( d \)-tubocurarine (Sigma). Drugs were dissolved in 0.9% NaCl solution, and all solutions were administered intravenously in volumes of 1 ml/kg.

Data are reported as the means±S.E.
The Student's t-test was used to evaluate data.
In rats anesthetized with pentobarbitone, arterial blood pressure and heart rate before drug administration was 114±1 mmHg and 415±5 beats/min (n=36), respectively. As shown in the left half of Fig. 1, 10 μg/kg guanabenz, 3 μg/kg clonidine, and 1 mg/kg guanethidine produced an initial rise in blood pressure followed by a delayed fall. Pressor responses began immediately after the termination of injection and reached a maximal rise within 2 min. The delayed depressor responses began within 5 min. Heart rate decreased in the initial phase and reached a plateau 5 to 10 min after drug administration. These changes of heart rate and the rise and fall in blood pressure were dose-dependent in the range of concentrations of guanabenz at 3, 10, and 30 μg/kg; clonidine at 1, 3, and

![Fig. 1. Effects of intravenous administration of guanabenz, clonidine, and guanethidine on mean arterial blood pressure (BP, upper half) and heart rate (HR, lower half) in pentobarbitone-anesthetized (left half) and pithed (right half) rats. The whole spinal cord of pithed rats was continuously stimulated at 2 Hz. Data shown are the mean±S.E. of the % change from the respective initial value (n=6 for saline, 4 for each agent). Each point is statistically significant, at least P<0.05, from saline except for the actions of guanabenz and clonidine on BP in pithed rats at 5, 10, and 20 min after administration.](image-url)
10 μg/kg; and guanethidine at 0.3, 1, and 3 mg/kg (n=4, at each dose).

When the spinal cord was continuously stimulated electrically at 2 Hz in atropinized pithed rats, blood pressure increased from 49±1 to 90±2 mmHg (n=16) and heart rate increased from 283±3 to 417±6 beats/min (n=16) within 3 min after initiation of stimulation, and then these parameters remained at constant levels for over 30 min (right half in Fig. 1). Under these experimental conditions, 10 μg/kg guanabenz and 3 μg/kg clonidine produced initial pressor responses corresponding to those seen in anesthetized rats, while the delayed depressor responses did not occur (upper half in Fig. 1). These results demonstrate that the pressor actions are of peripheral origin, probably due to vasoconstrictions induced by postsynaptic α-adrenoceptor stimulation (5, 6), since noradrenaline- and clonidine-induced pressor actions in pithed rats were selectively antagonized by prazosin, an α₁-adrenoceptor antagonist (6), and that the site of the depressor actions in anesthetized rats is not peripheral but central. One mg/kg of guanethidine, a peripheral adrenergic neuron blocking agent (7), produced a delayed depressor response in pithed rats which was similar to that seen in anesthetized rats. This finding indirectly supports the central origin of the depressor actions of guanabenz and clonidine. Our conclusion regarding guanabenz and clonidine is consistent with that obtained by many other investigators (2, 8, 9).

The basal heart rate maintained in pithed rats exactly corresponded to that seen in the anesthetized rats. Guanethidine at 1 mg/kg induced bradycardia, the degree of which was similarly marked, compared with that in the anesthetized rats (lower half in Fig. 1). This finding demonstrates that the bradycardia is of peripheral origin. On the other hand, clonidine-induced bradycardia has been mainly attributed to decreases in sympathetic discharges and increased vagal activities via the central structures (8, 9). It seems likely that guanabenz has a clonidine-like central inhibitory action on heart rate (1, 2, 10). However, in atria isolated from rabbits, guanabenz and clonidine inhibited positive chronotropic responses to sympathetic nerve stimulation. This inhibition by clonidine was completely antagonized by phentolamine; whereas a partial approx. 50% antagonism was seen against guanabenz, and cocaine prevented the phentolamine-resistant inhibition by guanabenz (3). As shown in the lower right part in Fig. 1, guanabenz and clonidine still decreased positive chronotropic responses of pithed rats to continuous spinal stimulation. The observations with clonidine in pithed rats are consistent with the findings of Drew (11). Furthermore, in the present experiments, the degrees of bradycardia elicited by guanabenz and clonidine in pithed rats were approx. 1/3 of the respective values in anesthetized rats. These results convincingly demonstrate that mechanisms of bradycardia induced by guanabenz and clonidine consist to some extent, approx. 2/3, of central components such as decreases in sympathetic discharges and increased vagal activities (1, 2, 8–10) and are also in part, approx. 1/3, attributed to inhibitory actions on peripheral adrenergic transmission such as a presynaptic α-receptor stimulating action for both agents and an additional guanethidine-like neuron blocking action for guanabenz (3, 6).

In conclusion, the inhibitory actions of moderate concentrations of guanabenz and clonidine on peripheral adrenergic neurons are partially involved in bradycardia but not in hypotension.

REFERENCES


