Central Sympathoinhibitory Action of a Direct-Acting Vasodilator, Budralazine, in Anesthetized Rats

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Abstract—Previous data on budralazine, 1-[2-(1,3-dimethyl-2-butenylidene)-hydrazino]-phthalazine, has indicated that it is a direct-acting vasodilating agent that does not produce marked tachycardia. The present study was undertaken to elucidate what effects may be seen on the central sympathetic nerve activity when budralazine is given systemically to rats. Budralazine (0.5, 1.0 and 5.0 mg/kg, i.v.) produced a dose-dependent reduction of mean arterial pressure. At doses of 0.5 and 1.0 mg/kg, budralazine induced bradycardia accompanied with a decrease in cardiac sympathetic nerve activity. Preganglionic adrenal sympathetic nerve activity was also reduced by budralazine (1.0 mg/kg, i.v.). A dose of 0.5 mg/kg of budralazine neither influenced carotid sinus nerve activity nor augmented aortic depressor nerve activity. On the contrary, a high dose of budralazine (5.0 mg/kg) produced simultaneous increases in the heart rate and cardiac sympathetic nerve activity along with a marked suppression of aortic depressor nerve activity. Plasma norepinephrine and epinephrine concentrations were also increased at a dose of 5.0 mg/kg. These findings suggest that budralazine doses of 0.5 and 1.0 mg/kg may reduce the sympathetic outflow that is mediated via central sympathoinhibitory action. Baroreceptor-mediated tachycardia occurred after high dose budralazine (5.0 mg/kg) administration in anesthetized rats.

Like hydralazine, budralazine (1-[2-(1,3-dimethyl-2-butenyldiene)-hydrazino]-phthalazine) (Fig. 1), which is a phthalazine derivative, exerts an effective hypotensive action due to its direct dilatation of vascular smooth muscle (1, 2). Although hydralazine-induced depressor action is accompanied with reflex tachycardia (3-5), it has been reported that budralazine is a long-acting antihypertensive drug that tends not to produce either reflex tachycardia in SHR (6) and renal hypertensive dogs (7) or essential hypertension in humans (8). Shibamura et al. (9) reported that budralazine possessed a weak agonistic action on presynaptic α-adrenoceptors. It was also demonstrated that budralazine produced a significant decrease in heart rate during the hypotensive phase in patients with essential hypertension (10). No reports are available in the literature concerning the effect of budralazine on cardiac sympathetic nerve activity. Moreover the mechanism of budralazine-induced bradycardia remains to be elucidated. Therefore, we were interested in the mechanism that causes bradycardia during the hypotensive phase of budralazine. Attention was focused on the following 3 points: the relationship...
Materials and Methods

General: Seventy-five normotensive male Wistar rats (320–380 g body weight and 9–12 weeks old) were used. Rats were anesthetized intraperitoneally with 500 mg/kg of urethane and 50 mg/kg of α-chloralose. After immobilization with gallamine triethiodide (10 mg/kg, i.v.), respiration was maintained through a tracheal cannula connected to a rodent respirator (Harvard Apparatus, model 683). Blood pressure and heart rate were monitored continuously from the left femoral artery with a pressure transducer (Nihon Kohden, MPU-0.5A). Drugs were administered intravenously through a catheter inserted into the left femoral vein. Rectal temperature was maintained between 36°C and 38°C with a heating pad.

Efferent nerve recordings: Inferior cardiac nerves, the cardiac sympathetic nerves, were isolated from the right stellate ganglion and cut near the heart under an operation microscope (WILD, M650). Inferior cardiac nerve activity (abbreviated as ICNA) was recorded from the central cut end of the nerve with bipolar platinum iridium wire electrodes. ICNA was amplified, passed through a filter (time constant of 0.001 and high cut filter of 1,000 Hz), displayed on an oscilloscope (Iwatsu, MS 5100A) and quantitated using a cumulative integrator (Nihon Kohden, EI-601G).

Adrenal nerves, branches from the pre-ganglionic splanchnic nerves, were dissected retroperitoneally and cut near the heart under an operation microscope (WILD, M650). Inferior cardiac nerve activity (abbreviated as ICNA) was recorded from the central cut end of the nerve with bipolar platinum iridium wire electrodes. ICNA was amplified, passed through a filter (time constant of 0.001 and high cut filter of 1,000 Hz), displayed on an oscilloscope (Iwatsu, MS 5100A) and quantitated using a cumulative integrator (Nihon Kohden, EI-601G).

Adrenal nerves, branches from the pre-ganglionic splanchnic nerves, were dissected retroperitoneally and cut near the adrenal gland. Adrenal sympathetic nerve activity (abbreviated as ASNA) was amplified and displayed as aforementioned. The amplified discharges were passed through a window discriminator (model 120, W-P instruments) which picked out discharges from background noise. The discharge rates of these nerves were counted every 1 or 10 sec by a real-time data analyzer (Nihon Kohden, ATAC-450) and displayed with a pen recorder. The nerve activity was expressed as % change of values with respect to the value before drug administration.

Afferent nerve recordings: The carotid sinus nerve and aortic depressor nerve were exposed and sectioned at their junctions with the glossopharyngeal and superior laryngeal nerves, respectively. Carotid sinus nerve activity (abbreviated as CSNA) and aortic depressor nerve activity (abbreviated as ADNA) were amplified and displayed using the same methods as used for the efferent nerve recordings. These nerve activities were also expressed as % change of values with respect to the value before drug administration.

Assay of plasma catecholamine concentrations: Blood (less than 3.0 ml) was drawn through a catheter inserted into the femoral artery of rats which received the same operation as that performed for the inferior cardiac nerve recordings. Plasma was immediately separated in a refrigerated centrifuge and stored at ~20°C. After purification with alumina, epinephrine and norepinephrine were separated by high-performance liquid chromatography (Shimadzu Seisakusho Co., Ltd.) (11). Epinephrine and norepinephrine concentrations were determined spectrofluorophotometrically using the trihydroxyindole method (Shimadzu, RF-500LCA) (12).

Baroreceptor denervation: The aortic depressor nerve and carotid sinus nerve were cut surgically. The denervation was judged complete if the sympathetic nerve activity was not inhibited during a norepinephrine-induced pressor response.

Drugs: Budralazine hydrochloride (Daiichi Seiyaku Co., Ltd., Tokyo) was dissolved in saline containing a 5% (v/v) polyethylene castor oil derivative (Nikkol CO-40TX, Nikko Chemicals) and 50% (v/v) ethanol. The volume injected was kept constant at 0.5 ml/kg b.w., and an equal volume of the vehicle was administered to the control rats.

Statistical analysis: Statistically significant differences were assessed by one way ANOVA followed by Duncan’s multiple range test (for multi-groups) or Student’s t-test (for two groups).

Results

Effect of budralazine on mean arterial
pressure in rats: Before treatment, the mean arterial pressure of the groups that received 0.5, 1.0 and 5.0 mg/kg doses of budralazine and of the group that received the vehicle were 122.7±10.1 (n=10), 131.8±6.7 (n=7), 118.8±9.5 (n=10) and 112.7±11.0 (n=10), mmHg, respectively. No statistically significant differences were noted between these pretreated values. Administration of vehicle was followed by a pressor response, after which the mean arterial pressure returned to the base line level. A dose-dependent reduction of mean arterial pressure was observed after budralazine administration (Fig. 2A).

Fig. 2. Effect of budralazine on mean arterial pressure (A) and heart rate change (B). Budralazine: (●) 0.5 mg/kg, (▲) 1.0 mg/kg, and (■) 5.0 mg/kg. Vehicle: (○) 0.5 ml/kg. Vertical lines show standard errors of the means. * indicates a significant difference (P<0.01) from the vehicle treated value.
The mean arterial pressure of the groups that received 0.5 and 1.0 mg/kg of budralazine returned to their approximate pretreated values 60 min after budralazine administration. However, the mean arterial pressure of the group that received 5.0 mg/kg of budralazine maintained its approximate maximum hypotensive response 60 min after drug administration.

Effect of budralazine on heart rate in rats: Before treatment, the heart rate of the groups that received budralazine doses of 0.5, 1.0 and 5.0 mg/kg and of the group that received the vehicle were 375.8±8.6 (n=10), 400.6±10.6 (n=7), 378.4±6.2 (n=10) and 399.3±9.5 (n=10) beats/min, respectively. No statistically significant differences were noted.

Fig. 3. Effect of intravenous budralazine on inferior cardiac nerve activity (ICNA) (A) and a specimen record (B). A: Budralazine: (●) 0.5 mg/kg, (▲) 1.0 mg/kg and (■) 5.0 mg/kg. Vehicle: (○) 0.5 ml/kg. Vertical lines show standard errors of the means. * indicates a significant difference (P<0.01) from the vehicle treated value. B: Traces from top to bottom are integrated (int.) inferior cardiac nerve activity (ICNA), discharge rate of ICNA, blood pressure (BP) and heart rate (HR).
between these pretreated values. Administration of vehicle was followed by a transient tachycardia, after which the heart rate returned to the baseline level. At doses of 0.5 and 1.0 mg/kg, budralazine produced significant decreases in heart rate: 10 min after drug injection, the changes were $-25.6 \pm 8.5$ and $-32.9 \pm 11.9$ beats/min, respectively (Fig. 2B).

On the other hand, a 5.0 mg/kg dose of budralazine did not produce any significant bradycardia; 30 min after injection, the heart rate increased by $45.1 \pm 15.3$ beats/min.

**Effect of budralazine on inferior cardiac nerve activity (ICNA) in rats:** Administration of vehicle was followed by a transient increase in ICNA, after which the ICNA returned to the baseline level. A 0.5 mg/kg dose of budralazine produced significant decreases in ICNA during the hypotensive phase (Fig. 3B). Ten min after 0.5 and 1.0 mg/kg doses of budralazine were administered, the ICNA decreased to 52.8 ± 11.6% and 41.9 ± 8.0%, respectively. A 5.0 mg/kg dose of budralazine, however, did not reduce the ICNA significantly. In fact, 30 min after administration, the ICNA was increased to 175.7 ± 30.7% (Fig. 3A).

**Relationship between the doses of budralazine and heart rate changes in rats:** As shown in Fig. 4, there was a significant correlation between ICNA and heart rate changes 30 min after budralazine administration ($r = 0.8602$, $P < 0.001$). Although 0.5 and 1.0 mg/kg doses of budralazine produced decreases in both ICNA and heart rate, a 5.0 mg/kg dose of budralazine increased both the ICNA and heart rate.

**Effect of budralazine on adrenal sympathetic nerve activity (ASNA) in rats:** Budralazine at 1.0 mg/kg produced a significant decrease in ASNA during the hypotensive phase (Fig. 5B): 30 min after administration, the ASNA was 50.5 ± 12.7% ($P < 0.01$ as compared with vehicle treated group) (Fig. 5A). Whereas 5.0 mg/kg of budralazine also reduced the ASNA, 30 min after administration the ASNA increased to 123.9 ± 30.7%.

**Effect of budralazine on plasma catecholamine concentrations in rats:** Thirty min after budralazine or vehicle administration, changes in plasma norepinephrine (NE) and epinephrine (E) concentrations were observed. Administration of 0.5 mg/kg of budralazine did not significantly change the NE and E concentration. On the contrary, 5.0 mg/kg of budralazine produced a significant increase in NE and E concentration (Table 1).

**Effect of budralazine on carotid sinus nerve activity (CSNA) and aortic depressor nerve activity (ADNA) in rats:** A 0.5 mg/kg dose of budralazine did not produce a significant change in CSNA as compared with the vehicle treated group. Thirty min after administration, the decrease in ADNA was not statistically significant as compared with the vehicle treated group (Fig. 6A). On the other hand, a 5.0 mg/kg dose of budralazine reduced the ADNA significantly (Fig. 6B); the ADNA decreased to 25.0 ± 7.1% maximally ($P < 0.01$ as compared with the vehicle treated group) (Fig. 6A).

**Effects of 5.0 mg/kg of budralazine on mean arterial pressure, heart rate and inferior cardiac nerve activity (ICNA) in buffer nerve-denervated rats:** Thirty min after a 5.0 mg/kg dose of budralazine was administered, changes in mean arterial pressure, heart rate and ICNA were observed in buffer nerve-denervated rats (Table 2).

In buffer nerve-denervated rats, budralazine (5.0 mg/kg) produced a decrease in
Fig. 5. Effect of intravenous budralazine on adrenal sympathetic nerve activity (ASNA) (A) and a specimen record (B). A: Budralazine: (▲) 1.0 mg/kg and (■) 5.0 mg/kg. Vehicle: (○) 0.5 ml/kg. Vertical lines show standard errors of the means. * indicates a significant difference (P<0.01) from the vehicle treated value. B: Traces from top to bottom are discharge rate of ASNA, blood pressure (BP) and heart rate (HR).

Discussion

The present study revealed that intravenous administration of budralazine (0.5–5.0 mg/kg, i.v.) produced a dose-dependent reduction of mean arterial pressure, and 0.5 and 1.0 mg/kg doses of budralazine produced a simultaneous decrease in both cardiac sympathetic nerve activity and heart rate in anesthetized rats. Moreover, intravenous administration of budralazine at a dose of 1.0 mg/kg decreased the preganglionic adrenal sympathetic nerve activity significantly. These findings suggest that the central action of budralazine may result in decreased sympathetic nerve activity and bradycardia. Shibamura et al. (9) reported that budralazine caused an
excitation of presynaptic \( \alpha \)-adrenoceptors in pithed rats. Although this may indicate that budralazine-induced bradycardia is partially due to its direct action on the heart, this excitation is weak and transient. The fact that budralazine also inhibited preganglionic adrenal sympathetic nerve activity indicates that the effect of budralazine at the sympathetic ganglia is negligible. Furthermore, carotid sinus nerve activity was not changed significantly by 0.5 mg/kg of budralazine. Although a slight decrease in aortic depressor
Table 1. Effect of budralazine on plasma catecholamine concentration in rats

|                     | Vehicle (n=6) | 0.5 mg/kg (n=6) | 5.0 mg/kg (n=5) |
|---------------------|--------------|----------------|-----------------|
| Norepinephrine (NE) | 176.3±39.8   | 82.8±11.4      | 451.0±168.4*§   |
| Epinephrine (E)     | 54.0±8.1     | 89.2±19.8      | 384.2±96.7*§    |

All values are expressed as pg/ml±S.E. * and § indicate a significant difference (P<0.01) from the vehicle and 0.5 mg/kg of budralazine treated groups, respectively.

Table 2. Effects of 5.0 mg/kg of budralazine on mean arterial pressure (MAP), heart rate (HR) and inferior cardiac nerve activity (ICNA) in buffer nerve-denervated rats

|                     | Vehicle (0.5 ml/kg, i.v.; n=6) | Budralazine (5.0 mg/kg, i.v.; n=6) |
|---------------------|-------------------------------|-----------------------------------|
|                     | Time  | MAP (%) | JHR (bpm) | ICNA (%) | Time  | MAP (%) | JHR (bpm) | ICNA (%) |
| PT                  |       | 123.5±2.0 mmHg | 376.2±11.4 bpm | —         |       | 115.8±5.5 mmHg | 381.9±15.7 bpm | —         |
| 0 min               |       | 100%    | 0         | 100%     | 0 min | 100%    | 0         | 100%     |
| 30 min              |       | 90.2±4.0% | 11.0±11.5 bpm | 107.3±13.4% | 30 min | 51.8±2.6%* | 11.1±9.6 bpm | 84.4±17.1% |

PT: pretreatment. All values are expressed as means±S.E. * indicates a significant difference (P<0.01) from the vehicle treated value.
nerve activity was observed with a budralazine dose of 0.5 mg/kg; this decrease was not significant as compared with the vehicle treated group (Fig. 6A). Because the aortic depressor nerve components are mostly baroreceptor afferent fibers in rats (13), the decrement of aortic depressor nerve activity is not due to chemoreceptor inhibition. Therefore, we excluded the possibility that a 0.5 mg/kg dose of budralazine produced a decrease in peripheral sympathetic outflow via its indirect actions such as the modification of the baro- or chemo-receptor reflexes. Our results firmly suggest that doses of 0.5 and 1.0 mg/kg budralazine may act centrally to reduce the peripheral sympathetic outflow. In this experiment, the fact that the initial hypotension induced by budralazine (0.5 mg/kg, i.v.) was not accompanied with the decreased cardiac sympathetic nerve activity leads us to suggest that this initial hypotension may be associated with the direct vasodilating action of budralazine.

Another important observation in the present experiment is that when a high dose of budralazine (5.0 mg/kg) was administered intravenously, mean arterial pressure decreased approximately 50% and aortic depressor nerve activity was reduced significantly. Moreover, budralazine-induced hypotension was accompanied with significant increases in cardiac and adrenal sympathetic nerve activity as well as tachycardia. The increased adrenal sympathetic nerve activity induced by a high dose of budralazine (5.0 mg/kg) coincided with an increase in plasma epinephrine concentration. However, in spite of the 5.0 mg/kg budralazine-induced excitation of sympathetic nerve activity, a decrease in blood pressure was observed. These results suggest that the hypotensive mechanism of budralazine is due to its direct action on the blood vessels. With regard to the relationship between sympathetic nerve activity and heart rate changes, our results suggest that inferior cardiac nerve activity may be responsible for the heart rate changes (Fig. 4).

In buffer nerve-denervated rats, a high dose of budralazine (5.0 mg/kg) did not increase cardiac sympathetic nerve activity or the heart rate. Our findings suggest that a baroreceptor-mediated reflex is associated with the increased sympathetic nerve activity and tachycardia induced by 5.0 mg/kg of budralazine.

In conclusion, the present findings suggest that budralazine doses of 0.5 and 1.0 mg/kg produce a decrease in sympathetic nerve activity and bradycardia mediated via its central action. However, it was demonstrated that baroreceptor-mediated tachycardia like that induced by hydralazine occurred after administration of a high dose of budralazine.

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