Centrally Applied Bombesin Increases Nerve Activity of Both Sympathetic and Adrenal Branch of the Splanchnic Nerves

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ABSTRACT—We reported that centrally applied bombesin probably excites both the gastric sympathetic and adrenomedullary systems and thus induces inhibition of gastric acid secretion. In the present study, therefore, we examined whether or not centrally applied bombesin directly affects sympathetic nerve activities in rats anesthetized with urethane. Intracerebroventricular administration of bombesin (0.3 and 3.0 nmol) increased discharge rates of the sympathetic branch as well as those of the adrenal branch of preganglionic greater splanchnic nerves. These effects were not secondary to changes in arterial blood pressure by bombesin. In conclusion, centrally applied bombesin directly activates both the sympathetic and adrenomedullary systems.

Keywords: Bombesin, Sympathetic nerve activity, Adrenal nerve activity

Bombesin given intracerebroventricularly (i.c.v.) produces a marked increase in the plasma level of adrenaline, while that of noradrenaline is slightly increased only at relatively large doses of the drug (1-3). As related to possible roles of bombesin in central regulation of gastric functions, bombesin is known to inhibit gastric acid secretion (4). In our previous study, we suggested that centrally applied bombesin probably excites both the gastric sympathetic nerves and adrenomedullary system and thus induces inhibition of gastric acid secretion, on the basis of following observations (5): The inhibition of gastric acid secretion induced by i.c.v. applied bombesin was not modified either by chemical sympathectomy with 6-hydroxydopamine or by bilateral adrenalectomy alone. However, this inhibition by bombesin was completely abolished by bilateral adrenalectomy plus chemical sympathectomy with 6-hydroxydopamine as well as by bilateral cutting of the greater splanchnic nerves.

There is, however, no direct evidence of the effects of bombesin (i.c.v.) on the splanchnic sympathetic nerves, except for a report by Somiya and Tonoue (6). Furthermore, the results reported by them were not conclusive; the adrenal sympathetic nerve was either stimulated or attenuated by bombesin.

Preganglionic greater splanchnic nerves ramify into two major branches: the adrenal branch and the sympathetic branch terminating in the coeliac ganglion. Therefore, in the present study, we examined whether or not centrally applied bombesin directly affects the nerve activity of the sympathetic nerves and the adrenal branches of the preganglionic greater splanchnic nerves in rats anesthetized with urethane.

Male Wistar rats weighing 400-450 g were maintained in a room at 22-24℃ under a constant day-night rhythm for 7-10 days and given food (laboratory chow, CE-2; Clea Co., Tokyo) and tap water ad libitum. Experiments were performed under urethane anesthesia (1.1 g/kg, i.p.). The femoral vein, the femoral artery and the trachea were cannulated for administration of drugs, arterial pressure recording and artificial respiration, respectively. The animal was then paralyzed with *d*-tubocurarine (1.5 mg/kg, i.v.) and artificially ventilated. The body temperature was monitored with a rectal thermometer and maintained at 36-37℃ by an automatically regulated heating pad. Animals were placed in a stereotaxic apparatus. Bombesin dissolved in saline was given into the lateral cerebral ventricle (AP: 7.5, L: 1.1, H: 3.5 below the surface of the brain) in a volume of 10 μl through a stainless steel micropipette (0.35-mm outer diameter).

One of the right adrenal branch or the sympathetic branch of the preganglionic greater splanchnic nerve was exposed through a right retroperitoneal flank incision. The central cut end of the nerve was placed on a pair of stainless steel wire electrodes. The entire preparation was immersed in a mixture of liquid paraffin and vaseline in order to provide electrical insulation and secure a good

recording of efferent nerve activity over several hours.

The efferent nerve activity was amplified by a bioelectric differential amplifier (model AB600G; Nihon Kohden, Tokyo) (band path 100-1000 Hz, -3 dB) and displayed on an oscilloscope (model 2G51; San-Ei Instrument Co., Ltd., Tokyo) and fed into the real-time UNIX signal processing computer (model 6450; Concurrent, Tinton Falls, NJ, USA). Nerve activity was analyzed and converted to the discharge rate by means of a software package "Laboratory Workbench" (Concurrent). The threshold level of the integration was set above the background noise level observed at postmortem.

Drugs used were: bombesin (Peptide Institute, Inc., Osaka); *d*-tubocurarine chloride, phenylephrine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA); sodium nitroprusside (Nacalai Tesque, Inc., Kyoto). Results are expressed as means±S.E. Dunnett's test was used for multiple comparison after one-way analysis of variance (ANOVA). Significant values are those with $P<0.05$.

As characteristically shown in Fig. 1, intravenous administration of sodium nitroprusside (100 μg/kg), a vasodilator, reduced arterial blood pressure and markedly increased discharge rates of the adrenal branch of the greater splanchnic nerve. On the other hand, intravenous administration of phenylephrine (80 μg/kg), an adrenergic α-adrenoceptor agonist, elevated arterial blood pressure and markedly suppressed discharge rates. Similar responses by administration of nitroprusside and phenylephrine were also observed in the nerve activities of the sympathetic branch. These results indicate that the splanchnic nerve activities were negatively affected by changes in arterial blood pressure.

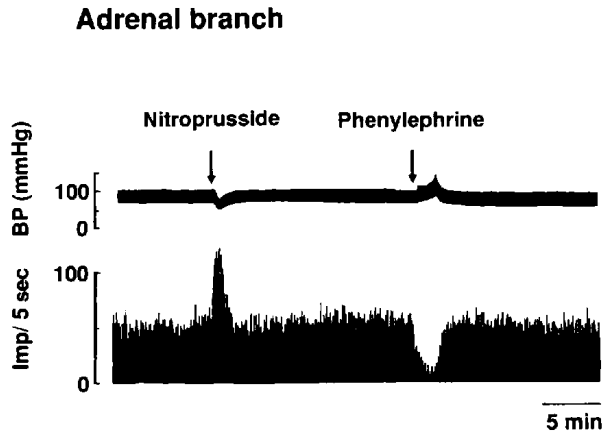

Fig. 1. Characteristics of adrenal nerve activity recorded in anesthetized rats. BP: arterial blood pressure. Phenylephrine (80 μg/kg, i.v.)-induced elevation of BP suppressed discharge rates of the adrenal branch, while nitroprusside (100 μg/kg, i.v.)-induced fall in BP increased discharge rates.

Bombesin (0.3 and 3.0 nmol) applied i.c.v. induced a marked and long lasting (at least 2 hr) increase in arterial blood pressure. The maximal response was observed between 20 and 80 min after the administration of this peptide (3 nmol), and the mean maximal increment was 35.4±4.8 mmHg (n=7). As shown in Fig. 2, administration of bombesin, i.c.v. in a dose of 3 nmol caused marked and long lasting increases in discharge rates of the sympathetic branch as well as that of the adrenal branch of the greater splanchnic nerves. These long lasting increases in discharge rates were correlated with those in blood pressure. On the other hand, intravenous application of bombesin at 3 nmol did not affect the discharge rates of the sympathetic and the adrenal branches and mean arterial blood pressure (data not shown). The increment of discharge rates in both branches reached a plateau about 90-150 min after the administration of bombesin, and this was followed by a gradual recovery. Discharge rates of the adrenal branch at 5, 30 and 90 min after the administration were 214±44, 320±23 and 546±96 percent of the preadministration values, respectively, while the values in the sympathetic branch were 184±20, 239±22 and 458±29, respectively. These effects of bombesin in inducing increased nerve activities of both branches were dose-dependent as shown in Fig. 3.

Circulating plasma catecholamines are thought to reflect sympathoadrenal activity (7, 8). In contrast to adrenaline, circulating plasma noradrenaline concentration can be elevated not only by an increased sympathetic nerve activity but also by an increased output of noradrenaline from the adrenal glands (9, 10). It is therefore likely that an increase in plasma level of noradrenaline is not always an index of activation of the sympathetic nerves.

As shown in Fig. 1, perception of a decrease in arterial blood pressure increased discharge rates of the adrenal branch, as a homeostatic manifestation. However, centrally applied bombesin increased the arterial blood pressure accompanied by parallel increases in discharge rates of the sympathetic branch as well as that of the adrenal branch of preganglionic greater splanchnic nerves. It is therefore evident that centrally applied bombesin directly, but not secondarily, affects discharge rates of both sympathetic and adrenal branches of splanchnic nerves. The present results support our previous indirect evidence that centrally applied bombesin probably excites both the gastric sympathetic nerves and adrenomedullary system and thus induces inhibition of gastric acid secretion (5).

Accumulating evidence suggests that there exist regional differences in the activities of sympathetic nerves in response to bombesin and some other neuroactive substances: decreased sympathetic outflow in the interscapular brown fat by bombesin, i.c.v. (11, 12), and reduced

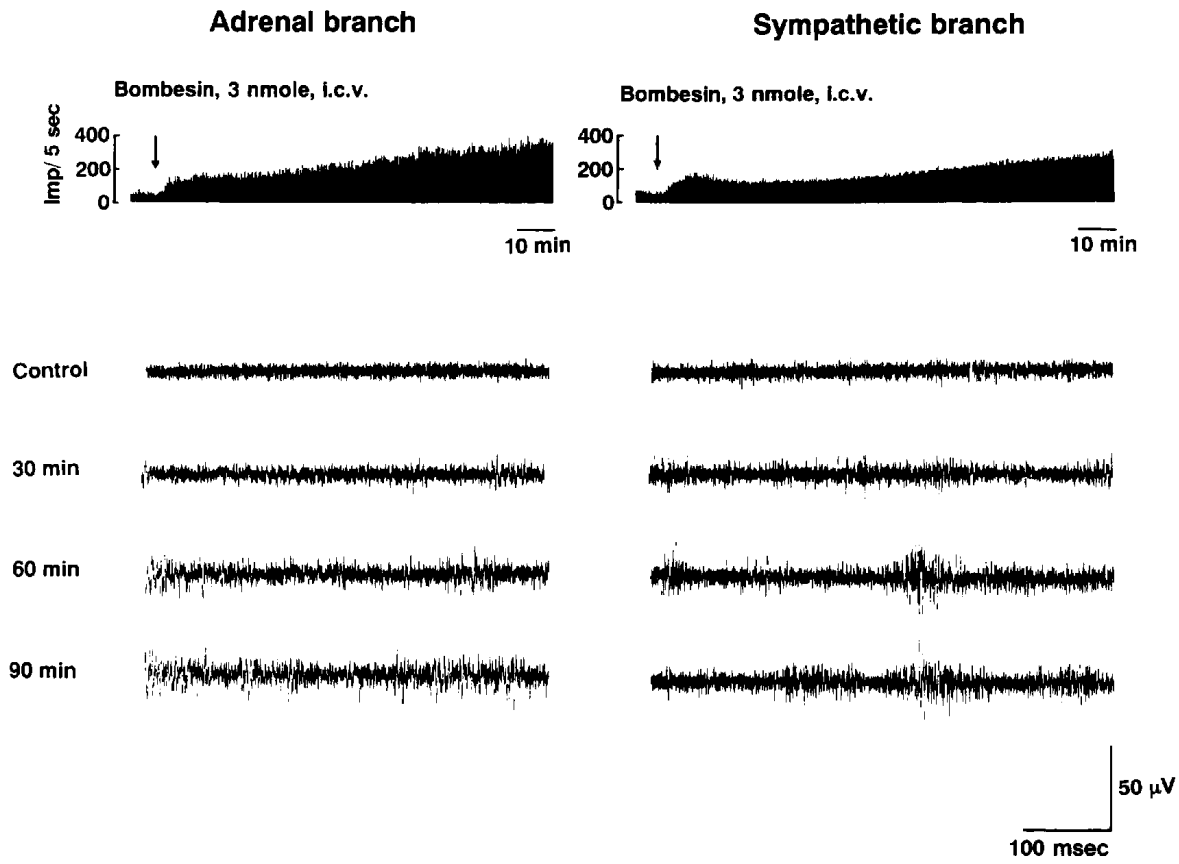

Fig. 2. The effect of bombesin (3.0 nmol, i.c.v.) applied on the discharge rates and action potentials of the adrenal (left panel) and the sympathetic branches (right panel) of the preganglionic greater splanchnic nerve. The original action potentials were drawn with a high speed thermal recorder.

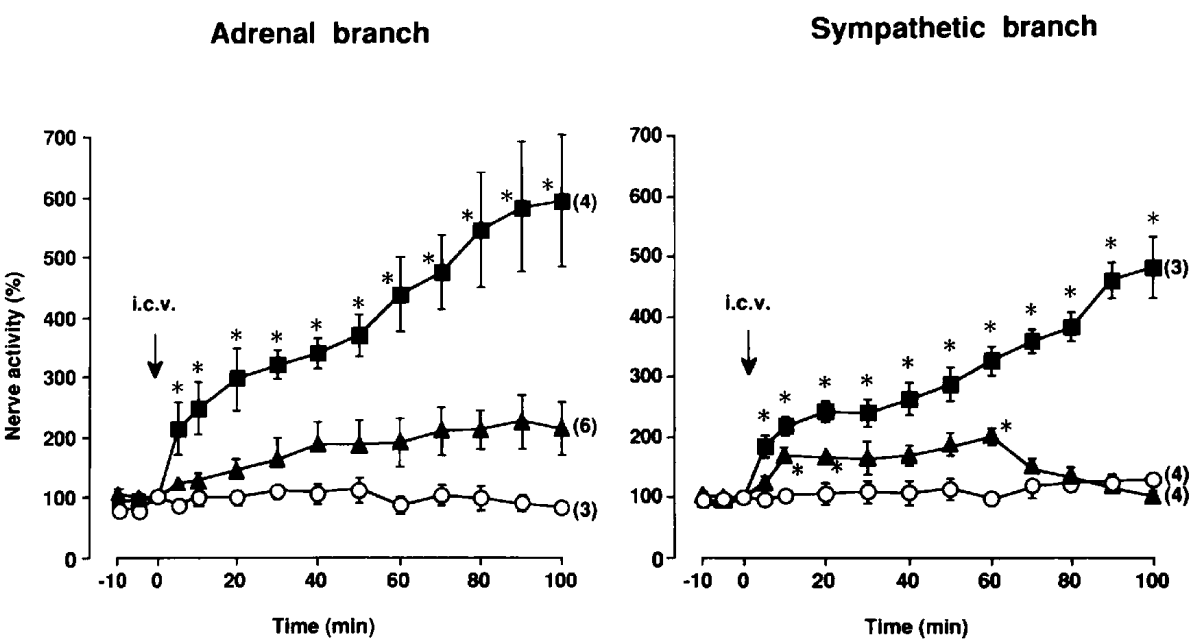

Fig. 3. Effects of bombesin i.c.v. applied on the discharge rates of the adrenal and the sympathetic branches of the preganglionic greater splanchnic nerve. Values are expressed as a percentage of preadministration values. ○: Vehicle (Saline); ▲: Bombesin, 0.3 nmol; ■: Bombesin, 3.0 nmol. Numbers in parentheses represent the number of experiments. *$P<0.05$, statistically significant difference from the respective control value with vehicle.

renal sympathetic nerve activity and increased cutaneous sympathetic nerve activity by prostaglandin E_2, i.c.v. (13). Furthermore, i.v. administration of interleukin-1β increased the activity of the adrenal and the splenic nerves, and it induced a transient increase followed by a long-lasting suppression in the renal nerve activity (14). Effects of central bombesin on the sympathetic neuron system in the other regions are therefore the subject of ongoing investigations.

In the present study, we demonstrate a direct evidence that bombesin centrally activates sympathetic nerves within the splanchnic regions as well as adrenal medullas.

Acknowledgments

This work was supported in part by a Grant-in-Aid for the Scientific Research No. 06670115 from the Ministry of Education, Science and Culture, Japan and a Grant from the Smoking Research Foundation, Japan. We thank Prof. emeritus A. Niijima, Department of Physiology, Niigata University School of Medicine for pertinent advice on the measurement of nerve activity and Mr. H. Takatsuji, Medical Research Laboratory, Kochi Medical School for his excellent technical assistance.

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