Note

Effects of Capsaicin Pretreatment on 2-Deoxy-D-glucose- and Acetylcholine-induced Catecholamine Secretion from the Adrenal Medulla

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Received April 21, 1989

A pungent principle of hot red pepper, capsaicin (CAP) is non-nutrient, but enhances energy metabolism *via* catecholamine secretion from the adrenal medulla¹⁾ through activation of the central nervous system.²⁾ We have recently found that CAP treatment of either neonatal or adult rats with pharmacological doses reduces the basal and CAP-induced epinephrine secretion from the adrenal medulla.³⁾ CAP treatments at pharmacological doses are known to cause selective impairment of primary afferent neurons.⁴⁾

This study was done to clarify the effects of CAP treatment on adrenal catecholamine secretion using 2-deoxy-D-glucose as a stimulant of adrenal sympathetic efferent nerves and acetylcholine as a direct stimulant of adrenal medulla. Though the degrees of effects are different, both neonatal and adult CAP treatments exert similar effects on basal and CAP-induced catecholamine secretion from the adrenal medulla.³⁾ Therefore, this study was done using only adult CAP treatment.

Capsaicin was obtained from Fluka AG (Buchs, Switzerland; Lot No. 258397 186). 2-Deoxy-D-glucose (2-DG) and acetylcholine chloride (ACh) were purchased from Nakarai Chem. Co. (Kyoto). Neostigmine bromide was obtained from Wako Pure Chem. Ind. Co. (Osaka). 3,4-Dihydroxybenzylamine hydrobromide (DHBA) was purchased from Aldrich Chem. Co. (Milwaukee, U.S.A.). All other chemicals were of guaranteed reagent grade.

Male Wistar rats (8 weeks old) weighing 220 ~ 250 g were used in all experiments. The rats were maintained on a

commercial stock diet (MF; Oriental Yeast Co., Tokyo) *ad libitum* in a temperature- (22 ~ 24°C) and moisture- (about 60%) controlled room lighted from 6:00 to 18:00, and were allowed to free access to drinking water. They were given a total dose of 310 mg/kg of capsaicin (10, 20, 30, 50, 100 and 100 mg/kg/day, sc) over 6 days under light ether anesthesia.³⁾ Capsaicin-treated rats were used in experiments 3 ~ 5 days after the last CAP treatment. Adrenal catecholamine secretion was measured as described previously.^{1~2)} Briefly, adrenal venous blood was taken continuously from the left adrenal gland for 15 min in 3 min fractions, under alpha-chloralose and urethane (75 mg/kg and 750 mg/kg, ip, respectively) anesthesia. The drugs were administered from the right femoral vein for 1 min. After centrifugation, DHBA was added to plasma as an internal standard. Sodium metabisulfite (10% solution) was also added in the proportion of 50 µl/ml plasma as a preservative. Plasma catecholamines were purified using activated alumina, and were assayed by high-performance liquid chromatography with electrochemical detection. Catecholamines were separated by a 5 µm C-18 column (Chemcosorb 5-ODS-H, 4.6 × 150 mm, Chemco Scientific

Fig. 1. Effects of Adult Capsaicin (CAP) Treatment on 2-Deoxy-D-glucose(2-DG)-induced Adrenal Epinephrine Secretion.

2-DG (250 mg/kg, iv) was administered for 1 min from time zero in normal (○) and adult CAP-treated (●) rats. The data are means ± SEM for 5 ~ 6 rats. Means not sharing a common super- or subscript letter are significantly different at $p < 0.01$.

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Abbreviations: CAP, capsaicin; 2-DG, 2-deoxy-D-glucose; ACh, acetylcholine chloride; DHBA, 3,4-dihydroxybenzylamine hydrobromide; sc, subcutaneously; ip, intraperitoneally; iv, intravenously; LHA, lateral hypothalamic area; NTS, nucleus tractus solitarius.

Co., Ltd., Osaka) with a mobile phase consisting of 10% methanol in 50 mM potassium phosphate buffer, pH 3.5, containing 10 μ M EDTA-2Na and 100 mg/l sodium 1-octane sulfonate. The flow rate was 1.0 ml/min. The detector (E-502 amperometric detector, Irica Instruments, Kyoto) potential was +750 mV versus the Ag/AgCl electrode.

All data are expressed as means $\pm$ SEM. After the data were converted into logarithms,⁵⁾ 2-way ANOVA⁵⁾ and Duncan's multiple-range test⁶⁾ were done.

The effects of 2-DG administration on adrenal epinephrine secretion in normal (untreated) and capsaicin-treated rats are shown in Fig. 1. In normal rats, 2-DG caused a significant increase in adrenal epinephrine secretion 6 min after administration. Subsequently, the secretion rates somewhat decreased. In capsaicin-treated rats, the basal epinephrine secretion rate decreased a little. The adrenal response to 2-DG was similar to normal rats by the 3~6 min fraction, but in the subsequent fractions, the response to 2-DG was fairly well suppressed.

To inhibit the degradation of acetylcholine, neostigmine

bromide (200 μ g/kg, iv) was administered 5~10 min before ACh infusion.⁷⁾ The use of neostigmine increased the basal secretion rate of adrenal epinephrine in both normal and capsaicin-treated rats (at 0 min in Fig. 1 and 2). ACh (12.5 μ g/kg, iv) caused a marked increase in epinephrine secretion from the adrenal medulla in normal rats. CAP treatment decreased the adrenal catecholamine response to ACh. But ACh produced a small augmentation of adrenal epinephrine secretion in CAP-treated rats.

It is thought that the nutritional conditions may change the responsiveness of rats to stimulants. But Cormareche-Leydier⁸⁾ and Castonguay and Bellinger⁹⁾ examined the effects of CAP treatment on food intake and body weight in adult rats and found that CAP treatment briefly affected feeding and body weight (the period in which a significant effect was found was about 3 days). Moreover, in our experiments, treatment with CAP under light ether anesthesia was done up to the 4th day, and thereafter, CAP treatment could be done without anesthesia. That is, we thought that the rats were desensitized to CAP by the 4th day of CAP treatment. 2-DG or ACh was administered at least 5 days after that. For these reasons, the endocrine responsiveness of rats evaluated in this study is thought not to be very much affected.

Insulin accelerates glucose use in the organs and consequently lowers blood glucose. It is thought that this drop of blood glucose is monitored centrally in the lateral hypothalamic area (LHA) and peripheral (hepatic portal and intestinal) glucose-sensitive units.¹⁰⁾ Insulin-induced epinephrine secretion from the adrenal medulla is thought to be due to the central hypoglycemia, and the peripheral hypoglycemia is supposed to lead to increased feeding.¹¹⁾ 2-DG, a nonmetabolizable analog of glucose, causes intracellular glucopenia and activates adrenal sympathetic efferent nerves.¹²⁾ 2-DG is known to act on the central nervous system (LHA and nucleus tractus solitarius (NTS) in the caudal brainstem) and the peripheral region.¹⁰⁾ On systemic administration, 2-DG is thought to cause a sympathoadrenal reflex through the activation of the caudal brainstem¹³⁾ because i) in decerebrated rats, in which the brain was cut between the hypothalamus and the caudal brainstem, a hypoglycemic response to 2-DG was still present,¹³⁾ ii) cervical transections of the spinal cord eliminated this response to 2-DG,¹⁴⁾ and iii) from the analogy of the action of insulin.⁹⁾ Furthermore, it is known that intracarotid application of 2-DG is more effective than intravenous infusion in producing hyperglycemia through secretion of adrenal catecholamine.¹⁵⁾ Taken together, 2-DG-induced adrenal nerve excitation is thought to be centrally mediated.

Khalil *et al.* have reported that insulin-induced adrenal catecholamine secretion is inhibited by neonatal CAP treatment.¹⁶⁾ In our preliminary experiments, adult CAP treatment also suppressed the adrenal response to insulin. The result of this study shows that 2-DG-induced adrenal catecholamine secretion was fairly well suppressed by CAP treatment. As mentioned above, insulin- and 2-DG-

Fig. 2. Effects of Adult Capsaicin Treatment on Acetylcholine Chloride(ACh)-induced Adrenal Epinephrine Secretion.

Neostigmine bromide (200 μ g/kg, iv) was administered 5~10 min before ACh injection. ACh (12.5 μ g/kg, iv) was infused for 1 min from time zero in normal (○) and adult CAP-treated (●) rats. The data are means $\pm$ SEM for 6~9 rats. Means not sharing a common super- or subscript letter are significantly different at $p < 0.01$.

induced activation of adrenal sympathetic nerves is thought to be centrally mediated. Further, in this and previous studies,³⁾ the basal rates of adrenal catecholamine secretion in CAP-treated rats were similar to that of splanchnicotomized rats (in the case of adults) and spinal rats (in the case of neonates), respectively.¹⁷⁾ It is believed that CAP treatment affects selectively primary afferent neurons,⁴⁾ but in addition, CAP may damage the efferent nerves to the adrenal medulla, from these results.

Takano *et al.* have reported that the reflex responses of the renal sympathetic nerve to baroreceptor or chemoreceptor stimulation is well maintained in neonatal CAP-treated rats.¹⁸⁾ But this study indicates that adrenal responsiveness to 2-DG was significantly lowered by CAP treatment in adult rats. Preliminary experiments showed that neonatal CAP treatment suppressed the augmentation of adrenal catecholamine secretion to 2-DG and the suppression was more significant than in the case of adult CAP treatment. Therefore, it may be possible that CAP treatment damages mainly nerve systems related to the adrenal medulla among sympathoadrenal systems.

In our previous study, the adrenal catecholamine contents were not very much affected by either neonatal or adult CAP treatment.³⁾ But this study demonstrates that the increases of adrenal catecholamine secretion rates in basal (stimulated by neostigmine) and ACh-induced states in normal rats were somewhat lowered by CAP treatment. From these results, it may be possible that ACh receptors on the adrenal medulla are partially damaged by CAP treatment.

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