PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 187, 370–374 (1988)

RAPID COMMUNICATION

EFFECT OF CAPSAICIN PRETREATMENT ON CAPSAICIN-INDUCED CATECHOLAMINE SECRETION FROM THE ADRENAL MEDULLA IN RATS#

TATSUO WATANABE, TERUO KAWADA, AND KAZUO IWAI*

Laboratory of Nutritional Chemistry,
Department of Food Science and Technology,
Faculty of Agriculture,
Kyoto University, Kyoto 606, Japan

Abstract. The effect of capsaicin pretreatment on adrenal catecholamine secretion induced by intravenously administered capsaicin was investigated in α-chloralose and urethane anesthetized rats. In neonatal vehicle pretreated rats, capsaicin (200 μg/kg, iv) caused a rapid and significant increase in adrenal epinephrine secretion, but neonatal capsaicin pretreatment (50 mg/kg, sc, on the 2nd day of life) significantly reduced both the basal and capsaicin-induced epinephrine secretion from the adrenal medulla. Adult capsaicin pretreatment (6 increasing doses/6 days, total 310 mg/kg, sc) also reduced both the basal and capsaicin-stimulated adrenal epinephrine secretion, though it was less effective compared with the neonatal capsaicin pretreatment. These results suggest the participation of capsaicin-sensitive neurons in capsaicin-induced catecholamine secretion from the adrenal medulla.
© 1988 Society for Experimental Biology and Medicine

Introduction. We have recently found that the dietary supplementation of capsaicin, a pungent principle of hot red pepper, in high fat diet-fed rats results in lowering of the perirenal adipose tissue weight (1), and that the effect of capsaicin is brought about by the enhancement of energy metabolism (2) through the secretion of catecholamine from the adrenal medulla (3,4). Intravenously administered capsaicin causes a marked increase in adrenal sympathetic efferent nerve activity. The adrenal catecholamine secretion induced by capsaicin is blocked by cholinergic blockers. Further, capsaicin does not cause catecholamine secretion from perfused isolated adrenal glands (submitted elsewhere). These results clearly suggest that the capsaicin-induced adrenal catecholamine secretion is mainly through activation of the central nervous system.

On the other hand, both neonatal and adult capsaicin pretreatment with pharmacological doses produces selective degeneration or neurotransmitter depletion of primary afferent peptidergic neurons (for a review, see ref. 5). The present study was performed to examine the participation of capsaicin-sensitive neurons in the capsaicin-induced adrenal catecholamine secretion, with both neonatal and adult capsaicin pretreatment.

Materials and Methods. Materials. Capsaicin was purchased from Fluka AG (Buchs, Switzerland; Lot No. 258397 186; for capsaicin pretreatment) and Sigma Chem. Co. (Saint Louis, MO; grade I; Lot No. 72F-06841; for measurement of the adrenal epinephrine response). All other chemicals were of guaranteed reagent grade.

Formation and metabolism of the pungent principle of Capsicum fruits, part XXI.

* To whom all correspondence should be addressed.

0037-9727/88 $1.50
Copyright © 1988 by the Society for Experimental Biology and Medicine.
All rights reserved.

Animals. Newborn Wistar rats were pretreated on the 2nd day of life with capsaicin (50 mg/kg, sc) (6) or the vehicle (0.9% NaCl solution containing 2% ethanol and 10% Tween 80). At the age of 9 - 12 weeks (240 - 320 g), the adrenal catecholamine response to capsaicin was examined in male rats. Male adult rats (220 -250 g) were given 6 consecutive daily doses (10, 20, 30, 50, 100 and 100 mg/kg, sc) of capsaicin under light ether anesthesia (7). The capsaicin-induced adrenal catecholamine response was also examined in these rats. As shown under Results, neonatal vehicle pretreatment, which is thought to have a greater effect than adult vehicle pretreatment, hardly affected the capsaicin-induced adrenal catecholamine secretion. Therefore, non-treated rats were used as controls for the adult capsaicin pretreatment.

Measurement of Adrenal Catecholamine Secretion. Adrenal venous blood collection was performed as described previously (4) with a slight modification. The rats were anesthetized with α-chloralose and urethane (75 mg/kg and 750 mg/kg, respectively). Respiration and the rectal temperature were maintained constant by means of an artificial respirator and a direct current heating pad, respectively. Adrenal venous blood was taken continuously from the left adrenal gland for 15 min. Capsaicin (200 μg/kg) was infused through the right femoral vein for 1 min. Afer the collection of adrenal venous blood, both sides of the adrenal glands in neonatal vehicle and capsaicin pretreated rats were extirpated, and then adrenal catecholamine was extracted with 0.4 N perchloric acid. Plasma catecholamine was purified using activated alumina. Both plasma and adrenal catecholamines were assayed by high-performance liquid chromatography with electrochemical detection (3, 4). We have recently reported that capsaicin causes the preferential secretion of epinephrine from the adrenal medulla (4). Therefore, only adrenal epinephrine secretion is dealt with in this report.

Statistical analysis. The significance of differences between means with the same variance and with different variances by the F-test was evaluated by Student's t-test and Aspin-Welch's test, respectively.

Results. In neonatal vehicle pretreated rats, capsaicin evoked a rapid and significant increase in epinephrine secretion from the adrenal medulla. This response was very similar to that observed in non-treated rats. On the other hand, neonatal capsaicin pretreatment resulted in a decrease in basal epinephrine secretion and little response of the adrenal medulla to capsaicin (Fig. 1). With the adult capsaicin pretreatment, both the basal and capsaicin-induced adrenal epinephrine secretions decreased, but the degrees of the reduction were small compared with those of neonatal capsaicin pretreatment (Fig. 2). The adrenal catecholamine levels in neonatal capsaicin and vehicle pretreated rats after the adrenal venous blood collection did not differ. (Table I)

Discussion. Both neonatal and adult capsaicin pretreatment reduced the basal and capsaicin-induced adrenal epinephrine secretion. For these reasons, it is thought that capsaicin pretreatment may deplete catecholamine from adrenal medulla. But the residual adrenal catecholamine after the adrenal venous blood collection did not differ.

The total secreted epinephrine and norepinephrine were about 2.5% and 0.3% (neonatal vehicle pretreated rats), and 0.5% and 0.1% (neonatal capsaicin pretreated rats), respectively. The actual adrenal catecholamine contents are expressed as the sum of the amounts of residual and secreted catecholamine. Even after these corrections, the adrenal catecholamine contents are not different between vehicle and capsaicin pretreated rats. These results show that adrenal catecholamine contents are not affected by capsaicin pretreatment.

Neonatal capsaicin pretreatment causes depletion of several putative neurotransmitters in the primary afferent neurons including substance P, vasoactive intestinal polypeptide, cholecystokinin and calcitonin gene-related peptide (5). Adult capsaicin pretreatment at least depletes substance P in the primary afferents (8). Intraarterial injection of capsaicin enhances the activity of primary afferent neurons (9). Substance P is a neurotransmitter of the small unmyelinated primary afferent neurons at the level of the spinal

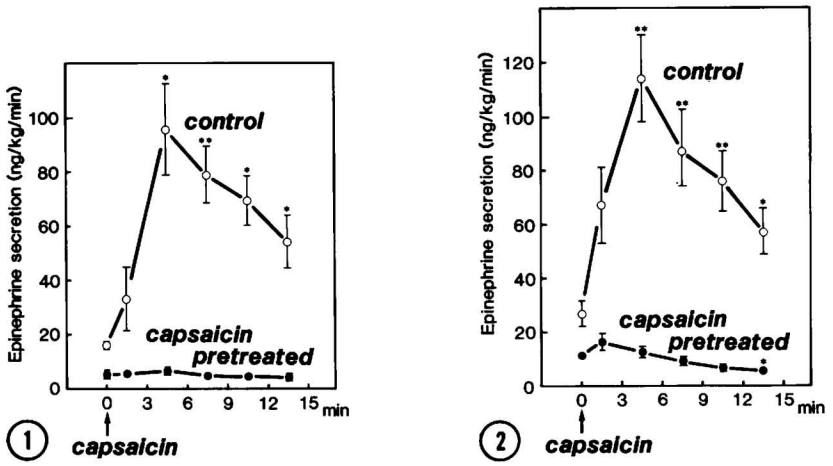

Fig. 1. Effect of neonatal capsaicin pretreatment on capsaicin-induced adrenal epinephrine secretion. Capsaicin (200 μg/kg, iv) was administered for 1 min from time 0 in neonatal vehicle (control, O) and capsaicin (●) pretreated rats. The values are means ± S.E.M. of the data for 4-6 rats. Asterisks indicate significant differences from basal values at time 0 ($^{*}p < 0.05$, $^{**}p < 0.01$). By paired analysis, all pairs except 0-3 min fractions are significantly different ($p < 0.05$).

Fig. 2. Effect of adult capsaicin pretreatment on capsaicin-induced adrenal catecholamine secretion. Capsaicin (200 μg/kg, iv) was administered for 1 min from time 0 in non-treated (control, O) and capsaicin pretreated (●) rats. The values are means ± S.E.M. of the data for 4-5 rats. Asterisks indicate significant differences from basal values at time 0 ($^{*}p < 0.05$, $^{**}p < 0.01$). By paired analysis, all pairs are significantly different ($p < 0.05$).

cord (10), and capsaicin causes substance P secretion from the spinal cord (11). Intrathecal injection of substance P at the level of the spinal cord causes adrenal catecholamine secretion (12). From these facts, capsaicin is thought to cause substance P release from the spinal cord by activation of the primary afferent neurons and/or the spinal cord, which activate the adrenal sympathetic efferent nerves and then enhance the adrenal catecholamine secretion. In this study, both neonatal and adult capsaicin pretreatment decreased the adrenal catecholamine secretion induced

Table I. Adrenal catecholamine contents in neonatal vehicle and capsaicin pretreated rats after the adrenal venous blood collection.

Neonatal pretreatment	Epinephrine (μg/gland)	Norepinephrine (μg/gland)	Dopamine (μg/gland)
vehicle(n=4)	11.9 ± 0.30	3.76 ± 0.20	0.138 ± 0.007
capsaicin(n=5)	13.1 ± 0.43	4.21 ± 0.21	0.096 ± 0.005*

The values are means ± S.E.M. Asterisk indicate a significant difference from vehicle pretreated rats ($p < 0.001$).

by intravenously administered capsaicin. The results suggest that such neurons as mentioned above participate in the capsaicin-induced catecholamine secretion from the adrenal medulla. As to sensory neurons, the effect of capsaicin pretreatment is more profound in neonates than adults (5). Therefore, the difference in the lowering effect on basal and capsaicin-induced adrenal catecholamine secretion of neonatal and adult capsaicin pretreatment may have been related to such actions as mentioned above.

In the splanchnic nerves and/or the adrenal medulla, some peptides exist (13-15). These peptides may modulate adrenal catecholamine secretion. Substance P inhibits or prolongs adrenal catecholamine secretion (16). Bombesin and vasoactive intestinal polypeptide enhance catecholamine secretion directly (17, 18). On the other hand, neonatal capsaicin pretreatment causes at least substance P depletion from the splanchnic nerves and adrenal glands (16). Capsaicin may elicit secretion of these peptides. But in the case of adrenal catecholamine secretion by capsaicin, the participation of these peptides is thought to be small, because capsaicin did not enhance catecholamine secretion from the perfused adrenal gland (submitted elsewhere).

These results clearly suggest that capsaicin-sensitive neurons, the function of which is impaired by the capsaicin pretreatment, are involved in the adrenal catecholamine secretion induced by intravenously infused capsaicin.

References

1. Kawada T, Hagihara K-I, Iwai K. Effects of capsaicin on lipid metabolism in rats fed a high fat diet. J Nutr **116:** 1272-1278, 1986.
2. Kawada T, Watanabe T, Takaishi T, Tanaka T, Iwai K. Capsaicin-induced β-adrenergic action on energy metabolism in rats: influence of capsaicin on oxygen consumption, the respiratory quotient, and substrate utilization. Proc Soc Exp Biol Med **183:** 250-256, 1986.
3. Watanabe T, Kawada T, Iwai K. Enhancement by capsaicin of energy metabolism in rats through secretion of catecholamine from adrenal medulla. Agric Biol Chem **51:** 75-79, 1987.
4. Watanabe T, Kawada T, Yamamoto M, Iwai K. Capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from the adrenal medulla of anesthetized rats. Biochem Biophys Res Commun **142:** 259-264, 1987.
5. Buck SH, Burks TF. The neuropharmacology of capsaicin: review of some recent observations. Pharmacol Rev **38:** 179-226, 1986.
6. Jancsó G, Kiraly E, Jancsó-Gábor A. Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature **270:** 741-743, 1977.
7. Obál F Jr, Benedek G, Jancsó-Gábor A, Obál F. Tail skin vasodilatation and bath test in capsaicin-desensitized rats. Pflügers Arch **387:** 183-188, 1980.
8. Jessell TM, Iversen LL, Cuello AC. Capsaicin-induced depletion of substance P from primary sensory neurones. Brain Res **152:** 183-188, 1978
9. Longhurst JC, Kaufman MP, Ordway GA, Musch TI. Effects of bradykinin and capsaicin on endings of afferent fibers from abdominal visceral organs. Am J Physiol **247:** R552-R559, 1984.
10. Otsuka M, Konishi S. Substance P - the first peptide neurotransmitter? Trends Neurosci **6:** 317-320, 1983.
11. Jhamandas K, Yaksh TL, Harty G, Szolcsányi J, Go VLW. Action of intrathecal capsaicin and its structural analogues on the content and release of spinal substance P: selectivity of action and relationship to analgesia. Brain Res **306:** 215-225, 1984.
12. Yashpal K, Gauthier SG, Henry JL. Substance P given intrathecally at the spinal T9 level increases adrenal output of adrenaline and noradrenaline in the rat. Neuroscience **15:** 529-536, 1985.
13. Linnoila RI, Diaugustine RP. Distribution of [Met^5]- and [Leu^5]-enkephalin-, vasoactive intestinal polypeptide- and substance P-like immunoreactivities in human adrenal glands. Neuroscience **5:** 2247-2259, 1980.
14. Kuramoto H, Kondo H, Fujita T. Substance P-like immunoreactivity in adrenal chromaffin cells and intraadrenal nerve fibers of rats.

Histochemistry **82**: 507-512, 1985.
15. Lemaire S, Chouinard L, Mercier P, Day R. Bombesin-like immunoreactivity in bovine adrenal medulla. Regl Pept **13**: 133-146, 1986.
16. Livett BG. The secretory process in adrenal medullary cells. In: Cantin M, ed. Cell Biology of the Secretory Process. Basel: Karger, p309, 1984.
17 Okubo M, Kaku K, Kaneko T, Yanaihara N. Effects of bombesin and gastrin releasing peptide on catecholamine secretion from rat adrenal gland, in vitro. Endocrinol Japon **32**: 21-27, 1985.
18. Malhotra RK, Wakade AR. Vasoactive intestinal polypeptide stimulates the secretion of catecholamines from the rat adrenal gland. J Physiol **388**: 285-294, 1987.

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 61470127 to K.I.) from the Ministry of Education, Science and Culture of Japan.

Received November 4, 1987
Accepted December 15, 1987