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Short communication

Selective activation of the sympathetic ganglia by centrally administered corticotropin-releasing factor in rats

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ABSTRACT

The sympathetic efferent pathway projects to the sympathetic ganglia and the adrenal medulla. In this study, we examined centrally administered corticotropin-releasing factor (CRF)-induced neuronal activation of noradrenergic postganglionic neurons in several kinds of the sympathetic ganglia (superior cervical, stellate and celiac ganglia) in anesthetized rats. CRF significantly increased c-Fos expression in the celiac and stellate ganglia, with more pronounced effect on the celiac ganglion. On the other hand, CRF had no effect on c-Fos expression in the superior cervical ganglion even at a higher dose. These results suggest that brain CRF selectively regulates neuronal activity of each sympathetic ganglion.

The sympathetic nervous system plays a critical role in the maintenance of homeostasis under physiological and pathophysiological conditions (Elenkov et al., 2000; Morrison et al., 2001). The sympathetic efferent pathway originates in the hypothalamus and the brainstem, and projects mono- and polysynaptically to the sympathetic preganglionic neurons located in the intermediolateral cell column (IML) of the thoracic spinal cord (Pyner and Coote, 2000). The sympathetic preganglionic neurons project to peripheral effector organs through postganglionic neurons in the sympathetic ganglia and to chromaffin cells containing adrenaline or noradrenaline in the adrenal medulla (Appel and Elde, 1988). Retrograde tracing studies have been shown that there appear to be functionally distinct subpopulations of preganglionic neurons based on location and cell size of these neurons (Grkovic and Anderson, 1996): preganglionic neurons residing in the IML at T1–T3, T1–T4 and T7–T11 innervate the superior cervical ganglion, stellate ganglion and celiac ganglion, respectively (Strack et al., 1989b; Jansen et al., 1995; Ranson et al., 1998).

Corticotropin-releasing factor (CRF) in the brain has been known to activate the sympatho-adrenomedullary outflow. Intracerebroventricularly (i.c.v.) administered CRF increases blood pressure, heart rate, blood glucose, plasma levels of noradrenaline and adrenaline, and sympathetic nerve activity (Brown and Fisher, 1984; Brown et al., 1985; Egawa et al., 1990; Croiset et al., 2000; Yokotani et al., 2001; Okada et al., 2003). We recently reported that CRF (1.5 and 3.0 nmol/animal, i.c.v.) induces neuronal activation of postganglionic neurons in the sympathetic celiac ganglion and chromaffin cells containing adrenaline in the adrenal medulla in rats (Yamaguchi-Shima et al., 2007). However, it remains unclear whether CRF in the brain activates ganglionic neurons in sympathetic ganglia other than the celiac ganglion.

In the present study, we examined the effect of centrally administered CRF on neuronal activity of noradrenergic postganglionic neurons in several kinds of the sympathetic ganglia (superior cervical, stellate and celiac ganglia). Because many previous studies have shown the colocalization of c-Fos (a marker for neuronal activation) and dopamine-β-hydroxylase (DBH; the enzyme converting dopamine to noradrenaline) to prove the neuronal activation in noradrenergic neurons in the brain and peripheral tissue (Temel et al., 2002; Rinaman, 2003; Yamaguchi-Shima et al., 2007), we used c-Fos to identify the neuronal populations activated by centrally administered CRF in the ganglionic noradrenergic neurons.

Male Wistar rats weighing approximately 300 g were used. Rats were purchased from SLC (Shizuoka, Japan) and were maintained in an air-conditioned room at 22–24 °C under a constant day–night rhythm for more than 2 weeks with food and water provided *ad libitum*. All rats were treated in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by Kochi University.

Under urethane anesthesia (1.2 g/kg, i.p.), intracerebroventricular administration of CRF and tissue sampling were performed as described in our previous papers (Yamaguchi-Shima et al., 2007). CRF (rat/human; Peptide Institute, Osaka, Japan) or vehicle (sterile saline) was slowly injected into the right lateral ventricle in a volume of 10 μl using a 10 μl Hamilton syringe. At 0, 60 or 120 min after the injection of CRF, the rats

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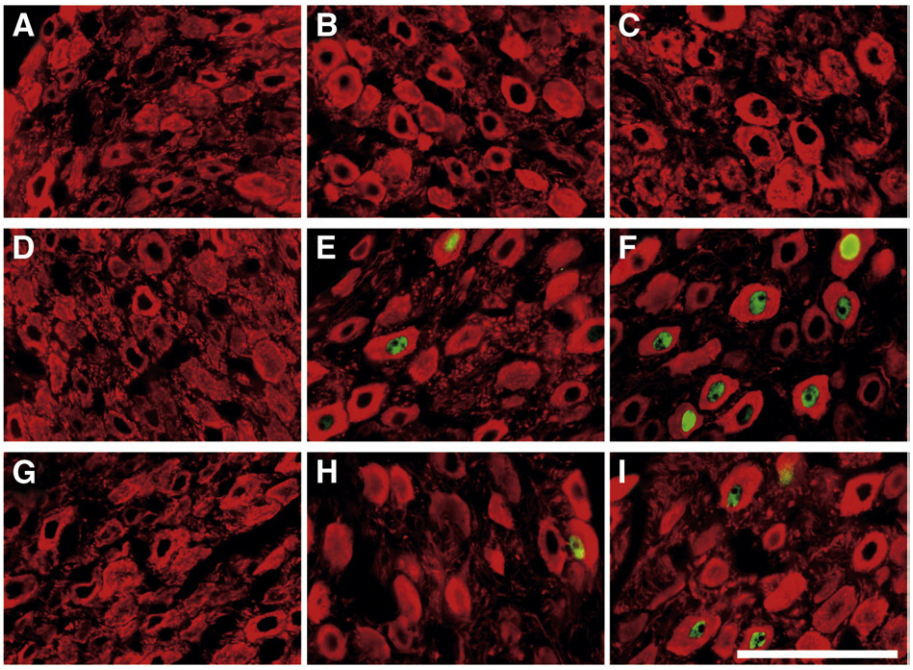

Fig. 1. Photomicrographs showing CRF-induced c-Fos expression in the sympathetic ganglia. Merged images show c-Fos- (green fluorescence) and dopamine-β-hydroxylase-immunoreactivities (red fluorescence) in the superior cervical ganglion (A, D, G), stellate ganglion (B, E, H), and celiac ganglion (C, F, I). CRF (1.5 and 3.0 nmol/animal) was intracerebroventricularly (i.c.v.) administered in anesthetized rats. (A–C) 0 min, (D–F) 60 min after administration of CRF (1.5 nmol/animal, i.c.v.), and (G–I) 120 min after administration of CRF (3.0 nmol/animal, i.c.v.). Scale bar = 100 μm.

were perfused through the left cardiac ventricle with 300 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Then, the sympathetic ganglia (superior cervical, stellate and celiac ganglia) were immediately removed and post-fixed overnight in the same fixative. After cryoprotection in 20% sucrose in 0.1 M phosphate buffer at 4 °C, frozen sections (20 μm-thickness) were cut on a cryostat.

Double immunofluorescence staining was performed as described previously (Yamaguchi-Shima et al., 2007). Briefly, sections were incubated in a cocktail of rabbit anti-c-Fos (1:500; Calbiochem, San Diego, CA, U.S.A.) and mouse anti-DBH (1:500; Chemicon, Temecula, CA, U.S.A.) antibodies for 4 h at room temperature. Then, the sections were incubated for 2 h at room temperature in a mixture of secondary antisera (1:200; Jackson ImmunoResearch Laboratories, West Grove, PA, U.S.A.); fluorescein isothiocyanate-conjugated goat anti-rabbit IgG and rhodamine-conjugated goat anti-mouse IgG. After these procedures, the sections were coverslipped using mounting medium VECTASHIELD (Vector Laboratories, Burlingame, CA, U.S.A.). Control experiments were performed by omitting primary antibodies as a test of cross-reactivity of secondary antibodies, and resulted in the absence of staining.

Photomicrographs were captured using a digital camera (DP70, Olympus, Tokyo, Japan) attached to a fluorescent microscope (AX70, Olympus). Quantification of the number of immunolabeled cells was carried out within a square of defined size (200 × 200 μm) placed on each section of sympathetic ganglia. The numbers of c-Fos-positive cells and ganglionic noradrenergic neurons were assessed by cell counting in 5 sections of each ganglion/animal. The percentage of c-Fos-positive cells in the total ganglionic noradrenergic neurons was calculated and expressed as the means ± S.E.M. The data were analyzed by one-way analysis of variance, followed by post-hoc analysis with the Bonferroni method for comparing a control with all other means. *P* values less than 0.05 were taken to indicate significant differences.

Double immunofluorescence staining showed subcellular localization of c-Fos in the nucleus as green fluorescence and DBH in the cytoplasm as red fluorescing.

It has been reported that central administration of CRF dose-dependently elevates blood pressure and plasma catecholamine (Fisher et al., 1982; Brown and Fisher, 1985; Fisher, 1989; Yokotani et al., 2001). Moreover, we previously showed that CRF (1.5 and 3.0 nmol/animal, i.c.v.) effectively induces neuronal activation in the adrenal medulla and celiac ganglion (Yamaguchi-Shima et al., 2007). Then, CRF was administered at doses of 1.5 and 3.0 nmol/animal (i.c.v.) in the present study. The administration of vehicle (10 μl saline, i.c.v.) had no effect on c-Fos expression in noradrenergic neurons in the sympathetic ganglia (superior cervical, stellate and celiac ganglia) (data not shown). Both doses of CRF (1.5 and 3.0 nmol/animal, i.c.v.) had no effect on noradrenergic neurons in the superior cervical ganglion throughout the experimental period (from 0 min to 120 min) (Fig. 1A,D,G, Table 1). On the other hand, the administration of CRF (1.5 and 3.0 nmol/animal, i.c.v.) induced c-Fos expression in noradrenergic neurons in the sympathetic stellate (Fig. 1B,E, H) and celiac ganglia (Fig. 1C,F,I). The CRF administration significantly increased the number of c-Fos-positive cells in the stellate and celiac ganglia (celiac ganglion ≫ stellate ganglion), throughout the experimental period (from 0 min to 120 min) (Table 1). Maximal responses were

Table 1
Effect of CRF on the percentage of c-Fos-positive cells in total noradrenergic postganglionic neurons in the sympathetic ganglia

	Superior cervical ganglion			Stellate ganglion			Celiac ganglion		
	0 min	60 min	120 min	0 min	60 min	120 min	0 min	60 min	120 min
CRF (1.5 nmol, i.c.v.)	0.98 ± 0.61	0.72 ± 0.25	0.60 ± 0.35	0.50 ± 0.25	15.79 ± 1.37*	11.78 ± 1.46*	2.01 ± 0.40	44.98 ± 5.73*	41.09 ± 4.55*
CRF (3.0 nmol, i.c.v.)	0.98 ± 0.61	0.92 ± 0.13	1.16 ± 0.11	0.50 ± 0.25	9.80 ± 1.53*	8.25 ± 1.25*	2.01 ± 0.40	31.54 ± 4.79*	27.98 ± 4.45*

Values are expressed as the means ± S.E.M. ($n = 4$ in each group).
*$P < 0.05$, significantly different from the respective baseline value (at 0 min).
i.c.v., intracerebroventricularly.