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Nerve fibers containing dynorphin A in cerebral arteries[1]

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ABSTRACT Nerve fibers containing dynorphin (Dyn) $A_{(1-17)}$-like immunoreactivity were identified around cerebral arteries of guinea pig. The immunoreactive nerve fibers were richly distributed in anterior and middle cerebral arteries, but sparsely in posterior cerebral and basilar arteries. Histofluorescent study showed that large and small cerebral arteries were abundantly innervated by monoamine nerve fibers. Pretreatment with 6-hydroxydopamine or reserpine reduced the concentration of Dyn A in the wall of arteries by about 60 % and 30 %, respectively. These results demonstrated that there exist Dyn A immuoreactive nerve fibers in cerebral arteries and Dyn A may coexist with monoamine in perivascular nerve fibers.

KEY WORDS cerebral arteries; immunohistochemistry; dynorphin; nerve fibers; biogenic monoamines; radioimmunoassay

Cerebral arteries are innervated by many peptidergic fibers[1-5], such as substance P (SP)[2], vasoactive intestinal polypeptide (VIP)[3], calcitonin gene-related peptide (CGRP)[4], neuropeptide Y (NPY)[5], and dynorphin (Dyn) B[6]. In the present study the distribution of Dyn A-containing fibers in cerebral arteries was examined.

MATERIALS AND METHODS

Distribution of Dyn A in cerebral arteries[7]

Guinea pigs, ♀ ($n=30$) weighing 200 ±s 50 g, were perfused under sodium pentobarbital anesthesia (50 mg·kg^{-1}, ip), with 300 ml of a solution containing 2 % paraformaldehyde and 0.25 % glutaraldehyde buffered with phosphate buffer 0.1 mol·L^{-1} (pH 7.4, 2—4 ℃ for 10 min. The cerebral arteries were stored in 0.3 % Triton-X 100 in phosphate-buffered saline at 2 — 4 ℃ for 4 d, and then reacted with anti-Dyn $A_{(1-17)}$ antibody (Sigma, diluted 1∶1000) in Triton-X phosphate-buffered saline at 2 — 4 ℃ for 2 — 3 d. They were treated with biotinylated goat anti-rabbit IgG (Vectastain ABC kit, Vector Laboratories Inc, 1∶100) at 20±2 ℃ for 3 h, followed by reagent A (avidin DH); reagent B (biotinylated peroxidas) for 3 h, and finally diaminobenzidine for 10 min. The specimens were dehydrated over night at 20±2 ℃, placed on coverslips, observed and photographed under a microscope (Nikon).

Cerebral arteries were incubated with normal rabbit antiserum or Dyn antibody which had been inactivated by excess Dyn for control.

Distribution of monoamine in cerebral arteries The glyoxylic acid technique (SPG method)[8] described by de la Torre, was used with some modifications to study the fluorescence of monoamine nerve fibers in cerebral arteries. Briefly, section was dipped 3 times (1 dip/s) in SPG solution. Sections were air dried for 20 min, heated for 5 min at 80 ℃ and coverslipped with mineral oil, and then heated on a hot plate for 90 s at 80 ℃. At last, sections were examined under fluorescence microscope.

Concentration of Dyn A in blood vessles Sprague-Dawley (SD) rats, ♀, weighing 250±s 42 g ($n=45$), were anesthetized with ether, perfused. The arteries were isolated and dissected and then dried, weighed at 22±2 ℃. The arteries of every 3 rats were collected as a single sample. The specimens were homogenized and centrifuged. The supernatants were stored at — 80 ℃ until radioimmunochemical assay of Dyn A_{1-13}[9].

Rats ($n=45$) were randomly divided into 3

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groups: 1. Normal saline (10 ml·kg^{-1}, ip) as a control. 2. Reserpine (1 mg·kg^{-1}, ip) 24 h before sacrifice. 3. 6-Hydroxydopamine (6-OHDA, 100, 20, and 20 mg·kg^{-1}, ip) for 3 successive before sacrifice.

Statistics Results were expressed in $\bar{x} \pm s$ and compared by t test.

RESULTS

Dyn A was visualized in guinea pig perivascular nerve fibers in cerebral arteries. Positive staining was seen predominantly in both single and fasciculated axons perpendicular to the long axis of the arteries. Immunoreactive fibers were present within anterior, middle, and posterior cerebral arteries as well as basilar artery. The density of immunoreactive fibers was highest in anterior and middle cerebral artery, lower density in posterior cerebral artery, and the least in basilar artery. (Fig 1, Plate 2)

Dyn A was also visualized in SD rat cerebral arteries ($n=5$).

The large and small cerebral arteries of guinea pig were richly innervated by monoamine (mainly NA) nerve fibers. There were no significant differences in density between arterior, middle, and posterior cerebral and basilar arteries. (Fig 2, Plate 3)

Immunoreactive Dyn A levels were measured in acid extracts of rat blood vessels. The concentrations of Dyn A-like immunoreactivity were high in renal vessels. Chemical sympathectomy with reserpine or 6-OHDA reduced the concentration of Dyn in systemic vessels by about 30 %, and 60 %, respectively. In thoracic aorta and aortic arch, the 6-OHDA-treated groups showed a decrease of Dyn A by 60 % and 63 %, respectively.

DISCUSSION

The present immunohistochemical studies revealed that various parts of the cerebrovascular bed investigated were all supplied with Dyn A-immunoreactive perivascular fibers. The distribution and density of Dyn A were similar to those of other neuropeptides found in cerebral blood vessels[1-6]. The density of Dyn A was less than that of monoamine nerve fibers, but their distribution patterns were similar. Chemical sympathectomy (by reserpine or 6-OHDA) could partially reduced the levels of Dyn A in blood vessels. These results suggested that some of Dyn A may coexist in part of monoamine perivascular nerve fibers and the others may exist in its own nerve fibers.

Because of the difficulty in isolating adventitia of systemic arteries from surrouding tissue and the high nonspecific binding of avindin and biotin on their thick wall, no definite results of immunohistochemistry on systemic arteries could be obtained.

Tab 1. Concentration of dynorphin A_{1-13} (ng/g wet wt) in arteries of rats under normal condition or after ip reserpine (1 mg·kg^{-1} 24 h previously) or 6-OHDA (100, 20, and 20 mg·kg^{-1} on previous 3 d). $n=5$, $\bar{x} \pm s$. $^{b}P<0.05$ *vs* **control.**

Group	Femoral artery	Aortic arch	Thoracic aorta	Abdominal aorta	Renal artery
Control	0.43±0.21	0.40±0.21	0.30±0.08	0.29±0.18	0.9±0.3
Reserpine	0.31±0.26	0.24±0.18	0.24±0.19	0.31±0.17	0.5±0.3
6-OHDA	0.26±0.21	0.15±0.06^{b}	0.12±0.07^{b}	0.16±0.07	0.76±0.08

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脑动脉上神经纤维存在强啡肽 A

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摘要 免疫组织化学方法显示豚鼠脑动脉上有含强啡肽 A_{1-17} 免疫样物质的神经纤维存在，大脑前和中动脉上分布最高。乙醛酸诱发荧光法显示有丰富的单胺能神经纤维分布在大的和小的脑血管上，大鼠预先给予6-羟多巴胺或利血平，血管壁上强啡肽含量明显下降。以上结果提示：血管壁上有强啡肽 A 纤维存在，并且它可能与单胺类神经递质共存。

关键词 脑动脉；免疫组织化学；强啡肽；神经纤维；生物源性单胺；放射免疫测定

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