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Modifications of the distribution of substance P-like immunoreactivity in the cerebral basal nuclei of the cat after electroacupuncture

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Summary. The distribution of Substance P in the fibrillar structures of the basal nuclei of the cat brain, and its modification with low frequency electroacupuncture (EA) stimulation, have been studied using the indirect immunocytochemistry technique. An increase in the immunoreactivity of Substance P (IR-SP) after stimulation with EA has been observed in the nucleus caudatus and nucleus putamen, in the globus pallidus, and in the amygdaloid complex. Abundant IR-SP fibres have also been seen in some areas of the internal capsule. These observations place the said IR-SP modifications in the basal nuclei of the cat brain, when subjected to low frequency EA.

Key words: Substance P, Electroacupuncture, Basal nuclei, Immunocytochemistry, Cat

Introduction

Electroacupuncture (EA) diminishes pain perception involving the met-enkephalin system (Vacca-Galloway et al., 1985; Muñoz, 1986; Vázquez et al., 1990). It has been observed that EA induces alterations in the distribution of met-enkephalin-like immunoreactivity in the cat thalamus (Vázquez et al., 1990). Substance P (SP) is a neuropeptide probably related to nociceptive transmission (Lembeck, 1953; Luque, 1988) and thus, with the met-enkephalin system (Hughes, 1975; Hökfelt et al., 1975; Jessel and Iversen, 1977). SP is localized in the small diameter fibres of the posterior horn of the spinal cord, which are considered to be involved in nociceptive transmission. Moreover, alteration of SP-immunoreactvity in the spinal cord after EA has been demonstrated (Vacca-Galloway et al., 1985). The cerebral basal nucleus shows SP-immunoreactive fibres in the striatonigral pathway (Glowinsky et al., 1982), the pallidus-caudate system (Florez and Martínez-Lage, 1983) and the amygdaloid complex (Bouras et al., 1986). In addition, it has been described that the injuries in the caudate nucleus abolish EA analgesia (Gonzalo, 1979; Takeshige et al., 1979).

Thus, in order to obtain new data on nociceptive transmission, the influence of EA on SP-immunoreactivity on the cerebral basal nuclei have been investigated.

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Materials and methods

Experimental procedure

Six adult cats (2-3 Kg) were divided into two groups: control group (three cats) and experimental group (three cats). Animals were anaesthetized with ketamine (50 mg/kg body weight) (Conrath et al., 1983); then, periferic stimulation with EA of low frequency was applied in Hegu and kumlun points, for 20 min. The frequency was 3-5 Hz and the intensity 8 mA. The correct stimulation was confirmed by the rhythmic contractions observed.

Tissue processing

Animals of both groups were perfused first with buffer (500 ml), and then, with 4% paraformaldehyde diluted in Sörensen buffer (Paese, 1962). The encephalon was then postfixed in the same fixative for 12 h and afterwards rinsed in several baths of sacarose in Sörensen buffer. After washing the encephalon was frozen in liquid nitrogen and 80 µm frontal sections were obtained in a cryostat.

Immunocytochemical staining

For the immunocytochemical detection of SP indirect techniques were used (Nakane and Pierce, 1966; Falini and Taylor, 1983; Conrath et al., 1986). Tissue sections were immersed in 0.3% H_2O_2 in methanol to eliminate endogenous peroxidase, then after hydration, sections were incubated with 1% normal sheep serum in 0.3% triton X-100. Sections were then incubated overnight with rabbit anti-SP antibody (1:1000, Cambridge Research Biochemicals, Cambridge, UK). As secondary

Table 1. Summary of SP-immunoreactivities.

		A11		A11.5		A12		A.12.5		A-13		A13.5		A14	
		control	EA	Control	EA	Control	EA	Control	EA	Control	EA	Control	EA	Control	EA
Cd	upper third	+	+	+	+	+	++	+	++	+	+	+	++	+	+
	middle third	+++	++	+++	++	+	++	+	++	+	++	++	++	+	+
	lower third	+++	+++	+++	+++	++	++++	++	+++	++	+++	++	+++	+	+
Fc		0	0	0	0	0	0	0	0	0	0	++	++++	++	+++
Put	upper third	+	++	+++	+++	++	+++	+	++	+++	++++	+	++++	-	++++
	middle third	+	+	+	+++	+	++	+	++	+	++	+	++++	-	++++
	lower third	++	++	++	++	++	+++	++	+++	++	+++	+	++	-	++++
GP		+++	++++	+++	++++	+++	++++	+++	++++	+++	++++	+++	++++	+++	++++
Ac		+	++/+++[a]	++	+++	+	+++	++	+++	++	++++	+	++	0	0
Ab		-	-	-	-	-	-	-	-	-	-	-	-	-	-
AI		+	++	++	++	+	++	+	++	++	++	++	++	+	++
Aco		++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	0	0
Am		0	0	0	0	++	+++	+	+++	++	+++	-	++	0	0
Aa		0	0	0	0	0	0	0	0	0	0	+	+++	+	+++
AL		++	++	++	++	++	++	+	++	++	++	++	++	0	0
Ci		-	-	-	-	++	++	-	+++	+++	++++	+++	++++	+++	++++
St		0	0	++++	++++	0	0	0	0	0	0	0	0	0	0

Intensity of staining: -, negative; +, very low; ++. low; +++, moderate; ++++, intense. 0: this structure is not present at this level. Aa: anterior amygdaloid region. Ab: nucleus amygdaloideus basalis. Ac: nucleus amygdaloideus centralis. Aco: nucleus amygdaloideus corticalis. AI: nucleus amygdaloideus lateralis. AL: ansa lateralis. Am: nucleus amygdaloideus medialis. Ci: internal capsule. Cd: caudate nucleus. Fc: fundus caudate. GP: globus pallidus. Put: nucleus putamen. St: stria terminalis. [a]: the lower staining was observed in the innner and outer thirds of the pars lateralis.

layer sheep anti-rabbit IgG horseradish peroxidase-conjugated antibodies were used at a dilution 1:250. Peroxidase was visualized with 3,3'diaminobenzidine. The following controls were used: a) pre-absorption of the first antibody with SP; b) omission of the different antibodies.

The mapping was carried out according to the stereotaxic atlas of Jasper and Ajmone-Marsan (1966).

Results

The results obtained are summarized in Table 1. Some of them have been illustrated in Fig. 1. Both in control and experimental groups SP-immunoreactivity

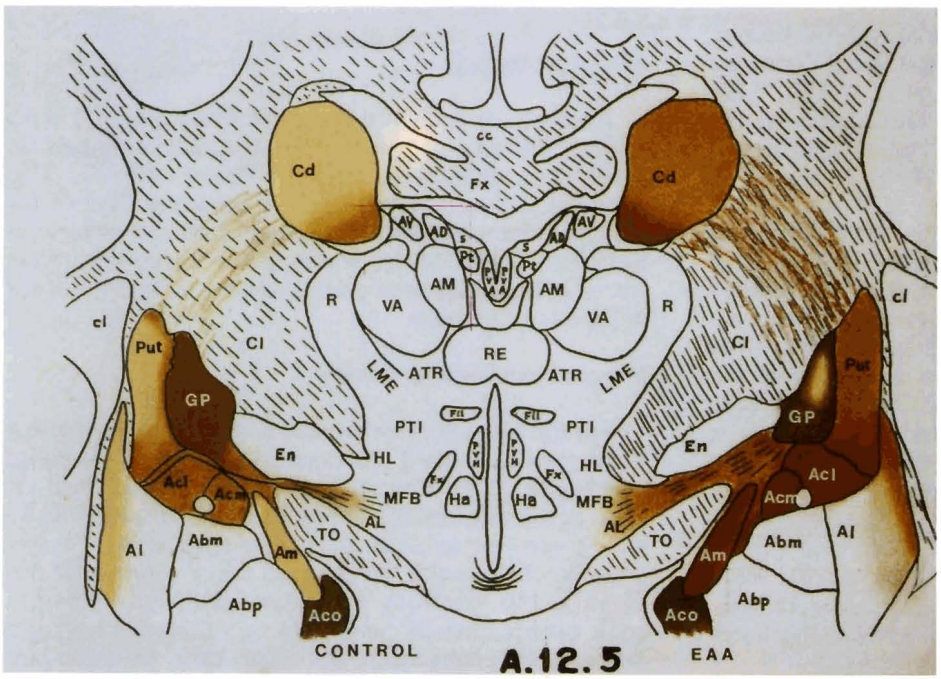

Fig. 1. Representation of the SP-immunoreactivity. On the left are represented the results obtained in the control animal; while the treated group is indicated on the right. The intensity of the brown colour indicate the staining intensity; four levels are observed; very low (i.e., Cd of the left), low (i.e., Cd of the right), moderate (i.e., Am of the right) and intense (Aco). Acl: nucleus amygdaloideus centralis (pars lateralis). Acm: nucleus amygdaloideus centralis (pars medialis). Aco: nucleus amygdaloideus corticalis. AI: nucleus amygdaloideus lateralis. Am: nucleus amygdaloideus medialis. Cd: caudate nucleus. GP: globus pallidus. Put: nucleus putamen.

was observed in fibres. No neuronal bodies were labelled by the antibody.

In the control group, immunoreactivity varied with the level at which the section was made. The upper third of the caudate nucleus stained very slightly while the other two thirds varied from moderate (more dorsal) to very low (more rostral) staining. The labelling of the fundus caudate was low. Immunoreactivity in the nucleus putamen greatly varied depending on the section; however the globus pallidus always showed a moderate staining. The ansa lenticularis, nucleus amygdaloideus centralis and lateralis showed a low or very low labelling, while the nucleus amygdaloideus basalis was always unreactive. The nucleus amygdaloideus corticalis was strongly labelled. The nucleus amygdaloideus medialis showed a low or very low staining, even being negative in A13.5 (Fig. 2a) region. When anterior amygdaloid region was observed, its labelling was very low. The internal capsule was reactive from A12 to A14 regions. The stria terminalis appeared in A11.5 showing a strong staining.

In the experimental animal some changes were observed (Tables 1, Fig. 1). The most usual was the increase in the intensity of staining; this increase was observed in some regions of the caudate nucleus and putamen, in fundus caudate, globus pallidus, nucleus amygdaloideus centralis, lateralis and medialis (Fig. 2), in ansa lateralis, internal capsule and stria terminalis. Usually, this increase was small (Table 1).

In summary, the most representative changes observed after EA stimulation was the increase of immunoreactive fibres in the caudate nucleus, putamen, globus pallidus and in the amygdaloid complex.

Discussion

Previously, in the corpus striatum, the presence of SP have been described in the strio-nigral pathway (Cuello et al., 1982) and in the upper nucleus caudate (Beach and McGeer, 1983; Bouras et al., 1986). This neuropeptide has also been found in the amygdaloid complex (Shakanaka et al., 1982). The stronger labelling observed has been located in the globus pallidus. A surprising datum is the absence of reactivity in neuronal body, in the caudate nucleus and putamen. This could be due to the stress of the animals; this stress could originate an unloading of several cerebral peptides, including SP. This stress condition was confirmed by the

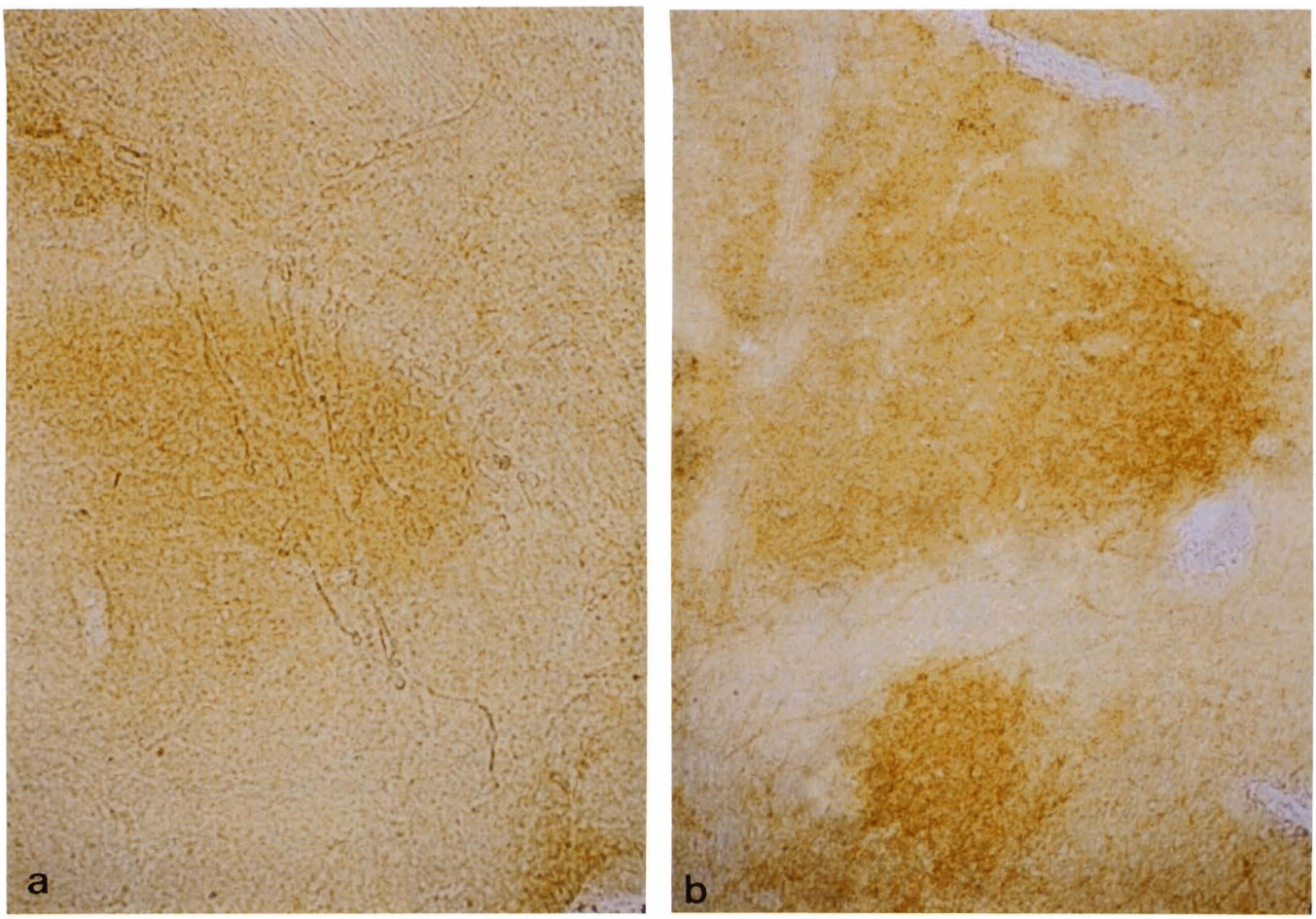

Fig. 2. a. Nucleus amygdaloideus medialis. Control animal. The immunoreactivity is very low. **b.** Nucleus amygdaloideus medialis. Experimental animal. Now immunoreactivity is moderate. x 250

hyperglycemia observed.

The existence of SP in the amygdaloid complex relate this nociceptive peptide (Jessel, 1982) to the emotional components of the limbic system; thus being, related to suffering.

It can be suggested that the SP-peptidergic system is antagonist of the met-enkephalinergic system (Vázquez et al., 1990) which is located in the structures studied. Thus, the existence of two systems could be indicated: one positive (analgesia, pleasure, etc) related to the met-enkephaline; and another negative (pain, suffering, etc) related with SP.

SP and corpus striatum have been previously implicated in the analgesic action of EA (Han et al., 1984; Vacca-Galloway et al., 1985; Ai et al., 1986; Luque, 1988). This possibility is confirmed with our results. The increase in the immunolabelling in the experimental animals would indicate that EA could prevent SP unloading, thus preventing nociceptive transmission. Therefore, low frequency EA would act on both antagonic and complementary peptidergic systems (met-enkephalin and SP) (Luque, 1988; Vázquez et al., 1990). EA would stimulate met-enkephalin unloading and would prevent that of SP; thus stimulating an analgesic system and preventing nociceptive transmission.

The increase of SP-immunoreactivity in the amygdaloid complex after EA treatment, can be interpreted as a blockade of the nociceptive transmission; since the cerebral amygdale is a part of the limbic system, that could also be implicated.

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