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Excitation of neurons in the nucleus locus coeruleus by substance P and related peptides

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Several lines of evidence suggest that the undecapeptide substance P (SP) is involved in synaptic transmission at the level of primary sensory neurons (nociceptive) and various other areas of the CNS[5,20,23]. The distribution of SP in the brain and spinal cord is very heterogenous[3], and there is histological and biochemical evidence for the existence of specific SP-containing pathways in the brain[13,16,22]. SP is present in nerve-ending enriched preparations and is released upon depolarization of intact neuronal tissue or synaptosomes[8,15,24].

SP has generally been found to be excitatory when applied iontophoretically at the vicinity of nerve cells. The areas investigated in this respect include the substantia nigra, the cortex (Betz cells), cuneate nucleus, spinal cord[7,11,12,19,26,29]. Its action has been consistently described as slow on onset and of long duration.

Recently Magnusson et al.[21] reported that an intracerebral injection of SP increases the turnover of brain noradrenaline. In addition, Hökfelt et al.[14] have reported that the locus coeruleus, which in the rat is composed almost exclusively of noradrenaline-containing cells[6], is densely innervated with SP-containing fibers. These findings prompted us to study the effect of this peptide on the single-cell activity of neurons in this nucleus.

Twenty-two male albino rats (230–260 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.) and prepared for recording as previously described[4].

Six-barrel electrodes were made as previously described[10]. Briefly, a single-barrel recording pipette (tip 1 μm) was glued alongside a conventional five-barrel micropipette (tip 15–25 μm) then filled with 2 *M* NaCl saturated with Fast Green (impedance 4–7 *M*). The distance between the tip of the recording electrode and that of the five-barrel micropipette was 15–25 μm. Fast Green was ejected at the end of the experiment to identify the recording site. One side barrel of the five-barrel micropipette was loaded with 4 *M* NaCl for automatic current balancing and the others with three of the following solutions: L-epinephrine bitartrate (Regis Chemical; 0.1 *M*, pH 4.0), L-norepinephrine bitartrate (Regis Chemical; 0.1 *M*, pH 4.0), SP (Beckman; 2.75 m*M*), physalaemin (Beckman; 2.6 m*M*), substance P 4–11 octapeptide (Beckman; 3.1 m*M*), eledoisin-related peptide (Sigma; 20 m*M*), neurotensin (gift of Dr. Carraway,

TABLE I

Sensitivity of neurons in the locus coeruleus to the iontophoretic application of substance P and other putative neurotransmitters

All peptides were applied for a maximum of two minutes using iontophoretic currents up to 115 nA — ACh, L-epinephrine, L-norepinephrine and met-enkephalin were effective in the 15–60 nA range.

	Locus coeruleus		
	Excitation	*No change*	*Inhibition*
Substance P	70	12	0
4–11 substance P	20	1	0
Physalaemin	17	0	0
Eledoisin peptide	6	2	0
Bradykinin	0	9	0
Neurotensin	0	5	0
Met-enkephalin	0	0	8
TRH	0	15	0
L-norepinephrine	0	0	8
L-epinephrine	0	0	9
ACh	22	5	0

Harvard Medical School; 3.0 m*M*), bradykinin triacetate (Sigma; 15 m*M*), met-enkephalin (Beckman; 6.5 m*M*), TRH (Beckman; 48 m*M*). All peptides except TRH were dissolved in 20 m*M* sodium acetate, pH 4.5 (acetate 20 m*M*, sodium 17.5 m*M*). The final pH was always between 4.2 and 5.0. TRH was dissolved in 40 m*M* sodium acetate pH 3.8, but bringing the pH down to 4 required the addition of a small amount of tartaric acid (0.1 *M*). It was inferred from the chemical formula of the peptides that, at the pH used, they should be positively charged and thus expelled with anodal (positive currents). This assumption was experimentally verified only in the case of substance P (Mroz, Guyenet, Aghajanian and Leeman, unpublished results).

Spontaneously active cells were recorded in the locus coeruleus or in the nearby mesencephalic nucleus of the fifth nerve whose cells are easily identified by their increased activity upon manipulation of the jaw.

SP (15–90 nA for 30–80 sec) excited most cells in the locus coeruleus producing a 30–300% increase over the basal firing rate (Table I and Figs. 1 and 2). Upon repeated application, its action was reproducible and did not exhibit any tachyphylaxis. The intensity of the response was related to the duration and intensity of the ejecting current. Iontophoretic currents of similar magnitude passed through a barrel containing only sodium acetate never altered the basal firing rate. The peak of the response occurred between 20 and 40 sec following the onset of the currents and total recovery usually required a minimum of one minute. These characteristics agree very closely with previous descriptions of the action of substance P in the CNS[19,26].

Physalaemin, substance P 4–11 octapeptide, and the eledoisin-related peptide (a synthetic hexapeptide) all share with SP a common *N*-terminal amino acid sequence; they have been shown by others to have SP-like activity in the periphery (gut and salivary glands) and the CNS[1,7,19,26]. The three peptides also activated locus coeruleus

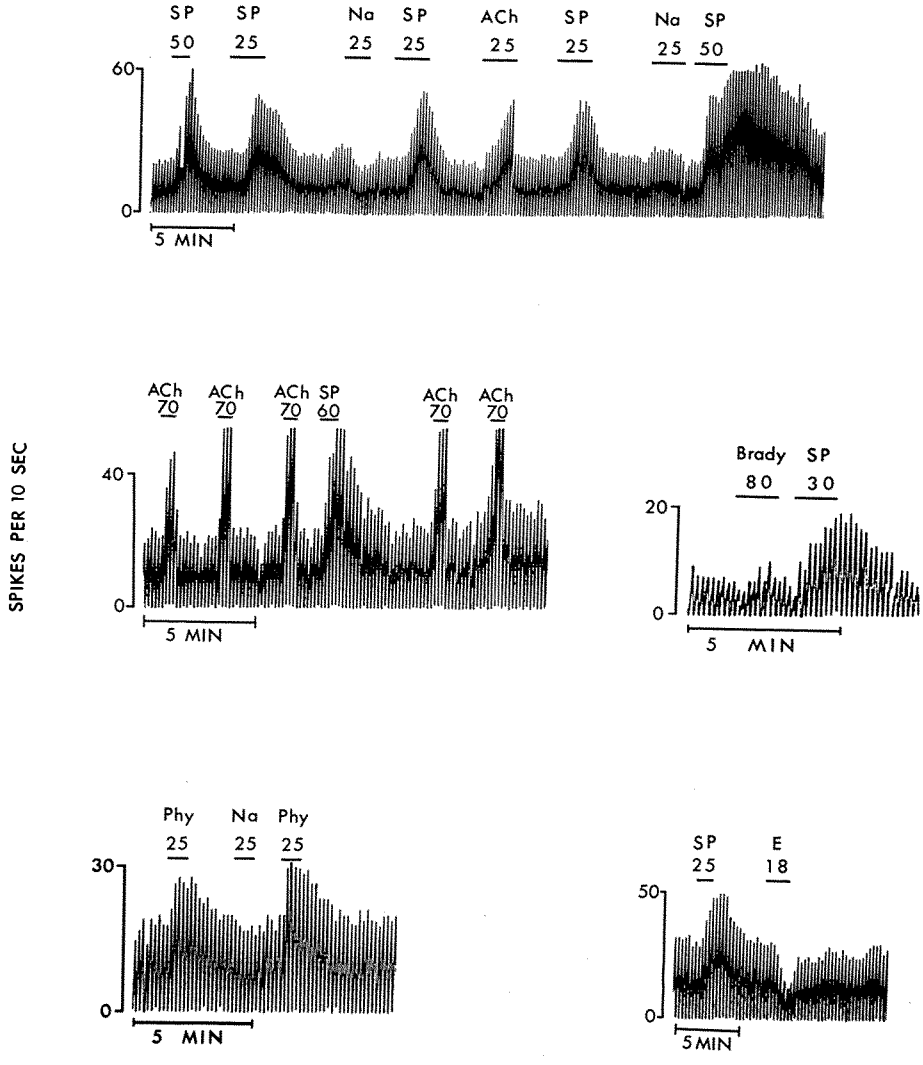

Fig. 1. Effect of substance P (SP), physalaemin (Phy), acetylcholine (ACh), bradykinin (Brady) and epinephrine (E) on the spontaneous activity of locus coeruleus cells. The bars above tracings indicate duration of microiontophoresis and numbers above bars refer to ejecting currents in nanoamperes (nA). The retaining current was 5 nA for the peptides and 20 nA for other compounds. Sodium acetate (Na) was used as a control.

cells in a way qualitatively similar to SP (Table I and Figs. 1 and 2). Although our experimental conditions do not permit definite conclusions to be drawn regarding their potencies relative to SP, physalaemin and the eledoisin-related peptide seemed to be roughly equipotent with SP at identical ejection currents. The onset of the activation produced by the 4–11 octapeptide was generally more rapid and recovery usually occurred much faster than with SP itself. This might indicate that as at the periphery the potency of the octapeptide is greater than that of the naturally occurring undecapeptide.

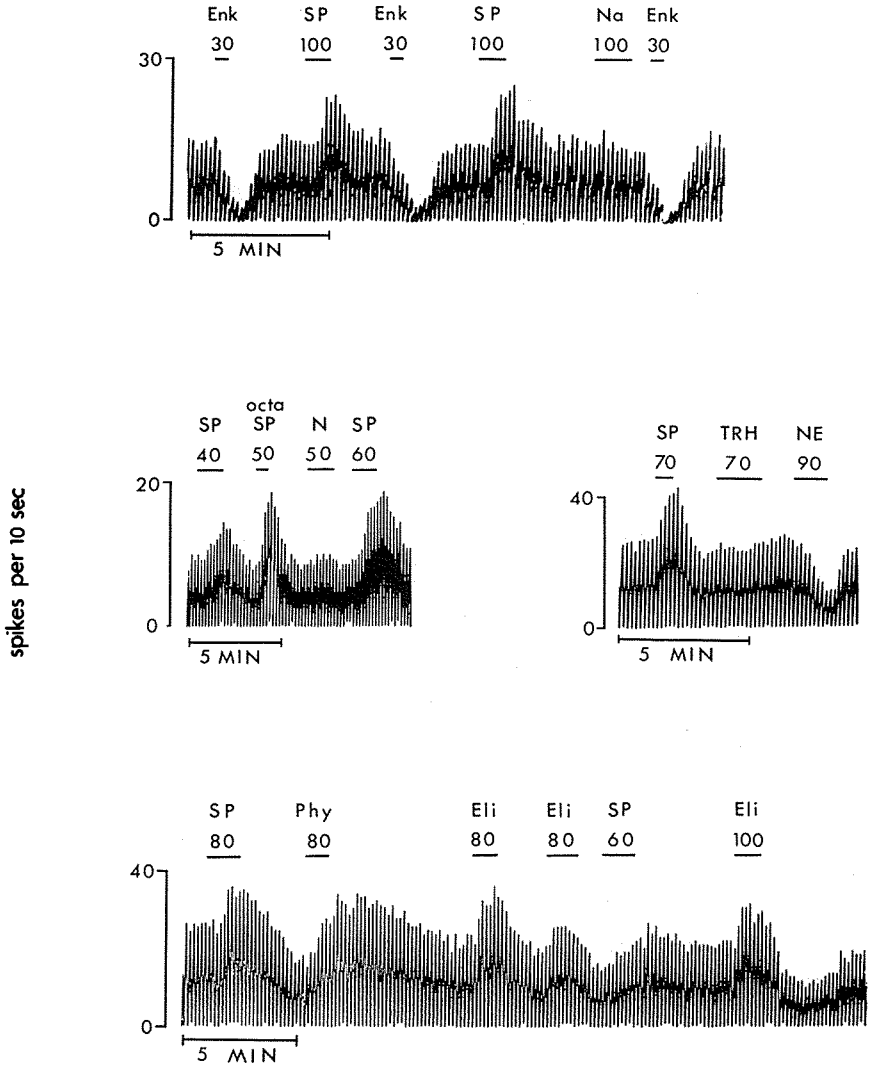

Fig. 2. Effects of substance P (SP), met-enkephalin (Enk), substance P 4–11 octapeptide (octa-SP), eledoisin-related peptide (Eli), physalaemin (Phy), neurotensin (N), TRH and L-norepinephrine (NE) on the spontaneous activity of locus coeruleus cells. The bars above tracings indicate duration of microiontophoresis and numbers above bars refer to ejecting currents in nanoamperes (nA). The retaining current was 5 nA for all peptides and 20 nA for the other compounds. Sodium acetate (Na) was used as a control.

The sensitivity of locus coeruleus neurons to ACh was found to decrease gradually from the ventricular surface to the deepest layers of the nucleus, where ACh-insensitive cells were eventually found (5 cells). These 5 cells could still be excited by SP. The result suggests that, in the locus coeruleus, there is no correlation between the sensitivity to ACh and SP contrary to what has been observed in the cortex[26].

Bradykinin was selected in this study because, like SP, it is a strongly basic peptide and was found to be active on cortical Betz cells and spinal mononeurons[17],

[26]. This peptide was totally inactive on locus coeruleus cells (the higher current tested was 100 nA for two minutes). Konishi and Otsuka[17] have already suggested that although both bradykinin and SP excite spinal motoneurons, their action depends on the presence of two distinct receptors. Our finding that bradykinin has no action on locus coeruleus neurons is consistent with their observation.

Neurotensin and TRH were also inactive on cells shown simultaneously to be activated by SP (Table I and Fig. 2). The presence of neurotensin in the locus coeruleus has never been investigated, but an extremely low density of TRH-containing fibers has been described in this area by Hökfelt et al.[12].

By contrast, the locus coeruleus is extremely rich in opiate receptors[25], and its sensitivity to the systemic or local application of morphine is well documented[2,18]. In agreement with a recent report (Scott-Young, III et al., in press), met-enkephalin was found to markedly depress the firing of locus coeruleus neurons (Table I and Fig. 2).

Previous experimenters have extensively studied the inhibitory action of L-epinephrine and L-norepinephrine on locus coeruleus neurons[4,28]. These two substances were routinely applied in the present work mainly to assess the quality of the pipette; they produced the expected depression on all locus coeruleus neurons tested (see Table I and Figs. 1 and 2).

Finally, SP and the three related peptides were found to be totally inactive on neurons located within the mesencephalic nucleus of the fifth nerve. This nucleus receives proprioceptive afferents. The fifth nerve nociceptive afferents terminate in the nucleus tractus spinalis nervi trigemini. Only this nucleus, homologous to the dorsal most laminae of the spinal cord was shown to contain massive concentrations of SP.

In conclusion, the present study shows that the great majority of neurons in the locus coeruleus are excited by the iontophoretic application of SP and related peptides. The specificity of this action is demonstrated by the fact that neither bradykinin, TRH nor neurotensin have any activity on these cells, nor have SP and its analogs any activity on neurons located in the mesencephalic nucleus of the fifth nerve. Moreover, the action of SP and met-enkephalin on the firing of locus coeruleus neurons is entirely consistent with the previous histological or biochemical demonstration of the presence of a dense network of terminals containing these peptides[14,25].

In the present study, the kinetics or the neuronal excitation induced by SP agree very closely with prior descriptions and would suggest that in contrast to met-enkephalin (Scott-Young, III et al., in press and present data) and probably TRH or LH-RH[9], SP is a slow acting transmitter. However, the question still remains whether the slow onset of its action corresponds to the real pattern of its postsynaptic action or is an artefact due to its delayed ejection from glass pipettes.

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