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COMPARATIVE STUDY OF SUBSTANCE P AND ITS FRAGMENTS: ANALGESIC PROPERTIES, EFFECT ON BEHAVIOR, AND MONOAMINERGIC PROCESSES

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Despite much evidence of the mediator or neuromodulator role of substance P in the CNS [6, 9] and data showing the broad spectrum of its action [10, 11, 15], it is not yet known exactly what are the functions of substance P in the body or how it interacts with other central ligands. It has also been postulated on the basis of data showing the neuromodulator action of certain C-terminal fragments of peptide hormones and their role in homeostatic processes of behavior [1, 2] that various fragments of substance P, its possible hydrolysis products, may also participate in the regulation of the behavioral and monoaminergic processes of the brain.

In the investigation described below a comparative study was made of the behavioral and analgesic effects of substance P and of certain of its fragments, and also of their effect on the content of biogenic monoamines (BM) in the rat brain.

EXPERIMENTAL METHOD

The peptides — substance P (SP) (H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) and its C-terminal fragments — a heptapeptide (SP 5-11), tetrapeptide (SP 8-11), and tripeptide (SP 9-11) — were synthesized at the Institute for the Study of Physiologically Active Substances (East Berlin); the dipeptide (SP 10-11) was synthesized at Warsaw University.

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TABLE 1. Analgesic Activity (in %) of Substance P and Its Fragments (by the "tail flick" test), Injected into the Cerebral Ventricles of Rats (M ± m)

Substance injected, dose, nanomoles		Initial response	Time after injection of substance, min			
			5	15	30	60
Control (physiological saline)		100,00±8,0	91,7±10,0	89,8±4,2	109,6±12,4	104,5±12,9
SP 1-11	5	100,0±8,9	104,8±10,9	127,8±14,5*	128,0±14,0*	82,8±8,2*
	25	100,0±9,7	41,8±5,9*	65,6±9,4*	99,7±12,0	115,7±8,3
	50	100,0±8,3	44,4±7,6*	68,2±9,6*	71,8±9,6*	77,7±9,6*
	100	100,0±4,0	147,0±22,6*	137,6±41,3*	128,5±43,3	89,0±12,3
SP 5-11	5	100,0±7,1	92,3±18,2	94,4±14,5	93,6±17,0	103,5±17,3
	25	100,0±9,7	64,1±19,2*	84,8±6,7*	73,6±8,6*	70,8±12,0*
	50	100,0±20,0	74,4±12,4*	105,2±17,9	80,3±10,4	78,1±9,4
	100	100,0±15,4	82,3±10,6	120,8±20,5	90,9±6,5	80,2±9,4
SP 8-11	5	100,0±6,25	87,6±8,0	130,0±9,6*	105,7±7,9	129,1±21,2
	25	100,0±2,6	89,0±11,5	74,5±4,9*	94,6±2,1	82,5±5,2*
	50	100,0±9,7	120,5±22,9	135,4±23,3*	131,3±23,2*	87,1±16,1
	100	100,0±7,1	111,5±17,3	143,6±7,1*	115,9±13,8	83,8±7,5*
SP 9-11	5	100,0±12,9	112,3±20,1	102,8±10,6	108,3±14,8	80,3±14,3
	25	100,0±10,0	77,8±4,6*	101,6±14,3	85,6±4,7*	108,5±8,1
	50	100,0±10,7	102,1±15,5	150,8±14,9*	116,5±22,1	99,3±10,1
	100	100,0±9,1	108,0±8,8	140,5±10,5*	151,3±24,5*	96,3±22,3
SP10-11	5	100,0±10,0	96,2±3,4	112,1±10,9	104,7±8,4	84,0±7,6
	25	100,0±10,3	71,4±4,3*	83,3±5,7*	77,5±12,5*	66,9±9,6*
	50	100,0±10,3	53,4±9,1*	110,8±6,1	91,6±11,5	93,5±8,5
	100	100,0±5,9	69,2±7,0*	85,7±6,5*	85,5±5,7*	54,6±4,6*

*$P \leq 0.05$ relative to control.

Male Wistar rats weighing 200-250 g were used. The test compounds were injected into the cerebral ventricles. The effect of SP and its fragments (SP 5-11, SP 8-11, SP 9-11) in doses of 2, 25, and 50 nmoles on behavior of the rats was studied under open field conditions; the number of separate behavioral responses was recorded in the course of 20 min and compared with data for control animals (receiving an injection of physiological saline). The rats were then decapitated and the content of BM — noradrenalin, dopamine (DA), serotonin (5-HT), and their metabolites homovanillic and 5-hydroxyindoleacetic acids — HVA and 5-HIAA respectively — in the brain was determined by a spectro-fluorometric method [4, 12, 13].

The analgesic properties of SP and its fragments were studied by the "tail flick" test [8] in doses of 5, 25, 50 and 100 nmoles. The latent period of the nociceptive response after injection of the peptides was calculated as a percentage of that before injection of the peptides.

EXPERIMENTAL RESULTS

Intraventricular injection of SP in doses of 5, 25, and 50 nmoles caused a dose-dependent increase in motor activity and depression of orienting activity in the majority of animals, with the appearance of behavioral responses such as head shaking, grooming, salivation (especially in a dose of 25 nmoles), and peripheral vasodilatation (hyperemia of the limbs, ears, and nose). Under the influence of SP in a dose of 5 nmoles no change was observed in the BM content; doses of 25 and 50 nmoles caused the 5-HT concentration to fall and the 5-HIAA concentration to rise; SP in a dose of 50 nmoles also affected the dopaminergic system: the HVA concentration was increased. An increase in the HVA concentration also was found after injection of SP into the substantia nigra [7], but our data do not agree with the stimulation of 5-HT secretion *in vitro* reported in the literature [14]. The fall observed in the 5-HT level was evidently due either to compensatory mechanisms with the acceleration of 5-HT metabolism *in vitro* or to the quantitative preponderance of SP produced as a result of administration of the exogenous substance, for we know that relations between SP and 5-HT in neurons are reciprocal in character [3].

A study of the analgesic properties of SP (Table 1) showed that 15-30 min after its injection in a dose of 5 nmoles the latent period of the nociceptive response was lengthened, but doses of 25 and 50 nmoles shortened the latent period. These results agree with data in the literature [5] on the appearance of hyperalgesia together with increased excitation in

response to high doses of SP. However, a further increase in the dose (100 nmoles) caused analgesia again.

SP 5-11 increased motor activity. Sniffing became more intensive than after injection of SP; grooming and exophthalmos were observed. By contrast with the action of SP, after injection of SP 5-11 salivation was weaker and peripheral vasodilatation was not observed. SP 5-11 increased the circulation of DA and 5-HT. The heptapeptide in a dose of 25 nanomoles shortened the latent period of the nociceptive response throughout the period of investigation, but in a dose of 50 nmoles it did so only 5 min after injection (Table 1).

The action of SP 8-11 differed from that of SP and SP 5-11. This fragment reduced motor activity and the intensity of sniffing by the rats, and transient salivation was observed. In a dose of 25 nmoles it increased the HVA level only, but in a dose of 50 nmoles it accelerated the circulation of BM and reduced the 5-HT concentration. An analgesic effect appeared 15 min after injection of SP 8-11 in doses of 5, 50 and 100 nmoles, but the effect of a dose of 50 nmoles was still present after 30 min.

SP 9-11 led to even more marked hypoactivity than SP 8-11. When injected in doses of 25 and 50 nmoles SP 9-11 accelerated the circulation of BM. The analgesic effect of SP 9-11 (Table 1) was exhibited on average 15-30 min after its injection in doses of 50 and 100 nmoles.

Dipeptide SP 10-11 in doses of 50 and 100 nmoles caused brief shortening of the latent period of the nociceptive response.

Comparison of the central effects of SP and its fragments showed qualitative changes in the animals' behavior and perception of the nociceptive response depending on the length of the peptide chain and the doses injected. For instance, the analgesic properties of SP (5 nmoles) changed into hyperalgesia (25 and 50 nmoles) and analgesia (100 nmoles). Hyperactivity and the dominant hyperalgesia of SP and SP 5-11 give way to hypoactivation and analgesia in SP 8-11 and SP 9-11. SP 10-11 is an algesic peptide.

SP fragments, like the whole molecule of the peptide, can simulate monoaminergic brain processes, mainly by accelerating the DA and 5-HT circulation and reducing the 5-HT concentration. It is suggested that endogenously formed fragments of substance P may play a functional role in the neurotransmitter regulation of behavior and nociceptive perception.

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