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Substance P, Hexapeptide pGlu^6(SP$_{6-11}$), Analgesia and Serotonin Depletion

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MÉSZÁROS, J., B. TARCHALSKA, S. GAJEWSKA, P. JANICKI, H. DURIASZ AND Z. SZRENIAWSKI. *Substance P, hexapeptide pGlu^6(SP$_{6-11}$), analgesia and serotonin depletion.* PHARMAC. BIOCHEM. BEHAV. 14(1) 11–15, 1981.— Substance P caused marked analgesic activity in rats after intraventricular administration and in mice after intraperitoneal injection. The hexapeptide pGlu^6(SP$_{6-11}$) was active in mice, but not in rats. Depletion of serotonin with p-chlorophenylalanine abolished the antinociceptive activity in mice, but not in rats, whereas lesion of raphe nuclei blocked the activity of substance P in the latter animals. Although different routes of administration were used, the results seem to indicate different mechanisms of analgesic activity of both peptides in rats and mice, as well as the different role of serotonergic transmission in pain control mechanisms in both species.

Substance P pGlu^6(SP$_{6-11}$) Analgesia Serotonin Rats Mice p-Chlorophenylalanine Raphe lesion

THE undecapeptide substance P (SP$_{1-11}$) is reported to exert marked analgesic activity when administered into the central nervous system in rats as well as after intraperitoneal injection in mice [3,7]. However, some authors do not support these observations [2]. Recently, in this laboratory the systemically injected SP$_{1-11}$ as well as its C-terminal hexapeptide analog, pGlu^6(SP$_{6-11}$) (PyroGlu) were found to induce analgesia in mice; the activity of the latter peptide in rats was not investigated [9].

The mechanism of analgesia after SP$_{1-11}$ related peptides is not clear. Some evidence, e.g., the antagonism by naloxone [7], suggest that a mutual link exists in both substance P and opiate induced antinociception, although direct action of these peptides on the opiate receptor is not very probable, their affinity being relatively low [10].

Serotonin (5-HT) is thought to play an essential role in opiate analgesia, the depletion of this amine resulting in the decrease or abolishing of the activity of opiates [8]. There is evidence that the SP$_{1-11}$ peptidergic system is related to serotonergic neurones. Significant amounts of SP$_{1-11}$ were found in the area of serotonergic raphe nuclei, and this peptide is present within some serotonergic nerve fibers [1]. The similarities between both opiate and SP$_{1-11}$ analgesia may then depend on their relation to the serotonergic system.

The purposes of the present study were: (1) to re-evaluate the analgesic activity of SP$_{1-11}$ given intraventricularly in rats and systemically in mice; (2) to determine the analgesic activity of PyroGlu given intraventricularly in rats; (3) to investigate the effects of 5-HT depletion by p-chlorophenylalanine as well as by electrolytic lesion of raphe nuclei on the antinociceptive activity of both peptides.

METHOD

Experiments on Rats

Male Wistar rats, 180–190 g body weight were used. p-Chlorophenylalanine (PCPA) methylester was injected IP, in three consecutive daily doses of 150, 100 and 100 mg/kg. Controls received saline in the same volume and on the same schedule. The last injection was made 24 hr before testing of analgesia. On this day, the animals were prepared for intraventricular injection. Under ether anaesthesia, the skin on the head was incised and a small hole in the skull bone made with a dental burr, 1 mm caudal from the bregma and 2 mm lateral to the midline.

Lesions of raphe nuclei were made under chloralhydrate anesthesia (120 mg/kg, IP) by DC anodic current 1.5 mA, passed for 15 sec from a stainless steel electrode, 0.2 mm diameter, insulated except at the tip. The electrode was introduced stereotaxically at coordinates A 0.4; L 0; and D −2.6 according to König and Klippel [4]. In sham controls, the electrode was introduced 0.5 mm above the mentioned point, other coordinates being the same, and retracted without passing of the current. During the surgery, the animals were prepared for intraventricular injection and tested for analgesia on the fifth day after the surgery. Pain thresholds were determined by means of the tail-compression method, as described elsewhere [11].

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The compression of the tail, necessary to evoke the vocalization of the animal, was read from micrometer scale and expressed in arbitrary units, ranging from 0–125, increasing in steps by 5 units. The limit of 125 compression units was never exceeded so as to prevent the mechanical lesion of the tail. The animals which did not vocalize were given the score of 130 units. Two control measurements were performed, and immediately after the second measurement the intraventricular injections made. Post-drug thresholds were determined 15, 30, 45, 60, 90, and 120 min after the administration of drugs.

Both peptides were administered in 0.37 nM doses (0.5 μg of SP_{1-11} and 0.275 μg of pyroGlu), dissolved in physiological saline containing 0.01 N acetic acid (pH 4.0). The volume of injected fluid was 5 μl. Siliconized glassware was used to prevent the adhesion of peptides to the glass. Controls received solvent in the same volume.

After the experiment, the animals were decapitated and their brains removed. In PCPA and saline-treated groups all forebrains were inspected for proper placement of intraventricular injection, and the animals showing any damage to the structures under- and/or overlaying the ventricle discarded. The forebrains of lesioned and sham-operated animals were randomly distributed into two lots: in the first, the level of 5-HT was determined, and in the second the placement of injection controlled. Remaining parts of these brains were taken for histological verification of placement of the lesion. Only these animals which showed small lesion in the area of ventral raphe nuclei without any marked damage to the lateral structures and/or central gray, were included into the results (Fig. 1). In these animals, a moderate but highly significant decrease of forebrain 5-HT levels was found (mean 342 ng/g of 5-HT vs 505 ng/g in sham controls, i.e., 33% decrease, $p<0.001$). A reference to the small group of animals with unsatisfactorily made lesions, having no change in 5-HT levels, will be made in the discussion.

The data obtained were analyzed by calculation of the arithmetical means and their standard errors for each group and each measurement. The significance between the means was estimated with Student's *t* test.

Experiments on Mice

White male Swiss mice, 22–24 g body weight were used. PCPA methylester was given IP during three consecutive days, 300 mg/kg daily, dissolved in normal saline. Controls received saline in the same volume.

Analgesia was tested with the hot-plate method ($t°55 \pm 0.5°$) 24 hr after last injection. Two parameters were estimated: the latency of hind-paw licking and the latency of jumping off.

SP_{1-11} was administered IP, in 185 and 370 nM/kg doses. PyroGlu was given in single 250 nM/kg dose. The effects were measured 60 min after administration of SP_{1-11} and 30 min after pyroGlu, according to the time of maximal activity of these compounds. Statistical analysis was made with the use of Student's *t* test.

RESULTS

Rats (Figs. 2 and 3)

The intraventricular injection of SP_{1-11} markedly increased pain thresholds in both saline pretreated and shamlesioned animals. The maximum effects were observed 15 min postdrug (172 and 150% of predrug values for saline and shamlesioned animals, respectively). The increase was significant statistically ($p<0.05$). In further measurements, the time-course of analgesia differed between both groups. In saline pretreated animals the pain sensitivity gradually returned to predrug values, and in the sham-operated rats there was rapid decrease of pain thresholds, with a marked decrease below predrug values, this decrease being statistically significant 45 and 60 min postdrug (nearly 50% of control values).

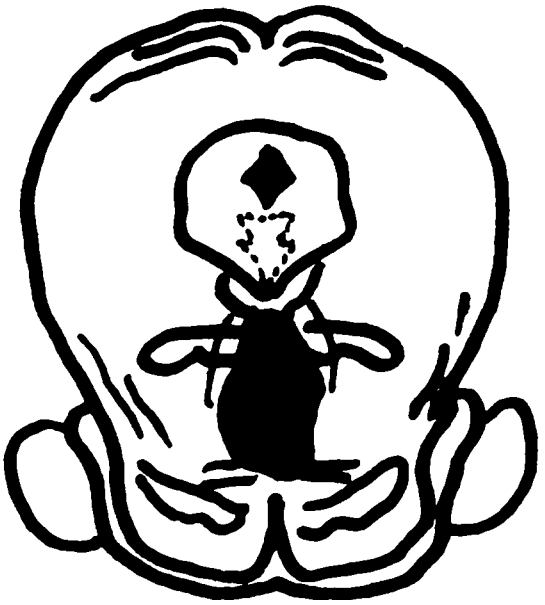

FIG. 1. Representative example of ventral raphe lesion. The blackened area shows the extent of tissue damage.

Pretreatment with PCPA did not change either the degree or the time course of SP_{1-11} analgesia. The absolute values of pain thresholds were strikingly similar to those obtained in saline-treated rats, even although PCPA alone slightly decreased the thresholds. Thus, after pretreatment with PCPA the mean percent increase of pain threshold caused by SP_{1-11} was even slightly higher than after the peptide alone.

Lesion of raphe nuclei abolished completely the analgesic activity of SP_{1-11}. However, the hyperalgesia was not abolished, and was even slightly greater than in sham-lesioned animals.

PyroGlu, administered intraventricularly in doses equimolar to those of SP_{1-11}, did not cause any changes in pain thresholds, either in controls or after 5-HT depletion.

Mice (Fig. 4)

Intraperitoneal injections of both SP_{1-11} and pyroGlu increased significantly the pain thresholds. The latency of hindpaw licking increased up to 182 and 194% of control values after higher and lower doses of SP_{1-11}, respectively, and to 190% of these values after pyroGlu. The latency of jumping-off increased, respectively, up to 203, 206 and 142% of controls.

Pretreatment with PCPA abolished completely the analgesic activity of both peptides in doses employed. The latency of hindpaw licking after SP_{1-11} in PCPA pretreated mice was even somewhat shorter than in controls (82 and 75%, respectively, after higher and lower dose), but the difference was not significant. The latency of jumping-off

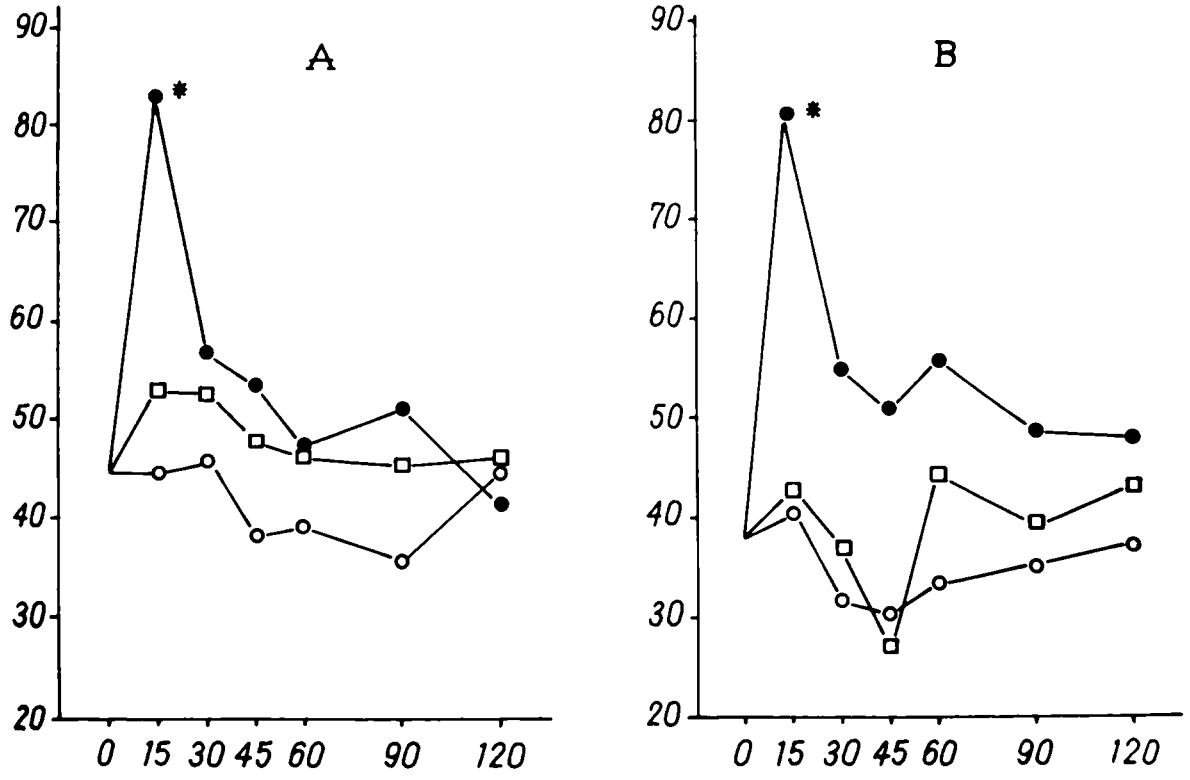

FIG. 2. The effect of PCPA pretreatment on analgesic action of intraventricular SP_{1-11} and PyroGlu in rats. Tail compression test. Panel A: pretreatment with saline. Panel B: PCPA pretreatment. Abscissa: pain threshold in arbitrary units. Ordinate: time in minutes. Administration of drugs immediately after time 0. Full circles: SP_{1-11}; open circles: PyroGlu; open squares: solvent. An asterisk denotes significant difference in respect to intraventricular solvent in appropriate time ($p<0.05$).

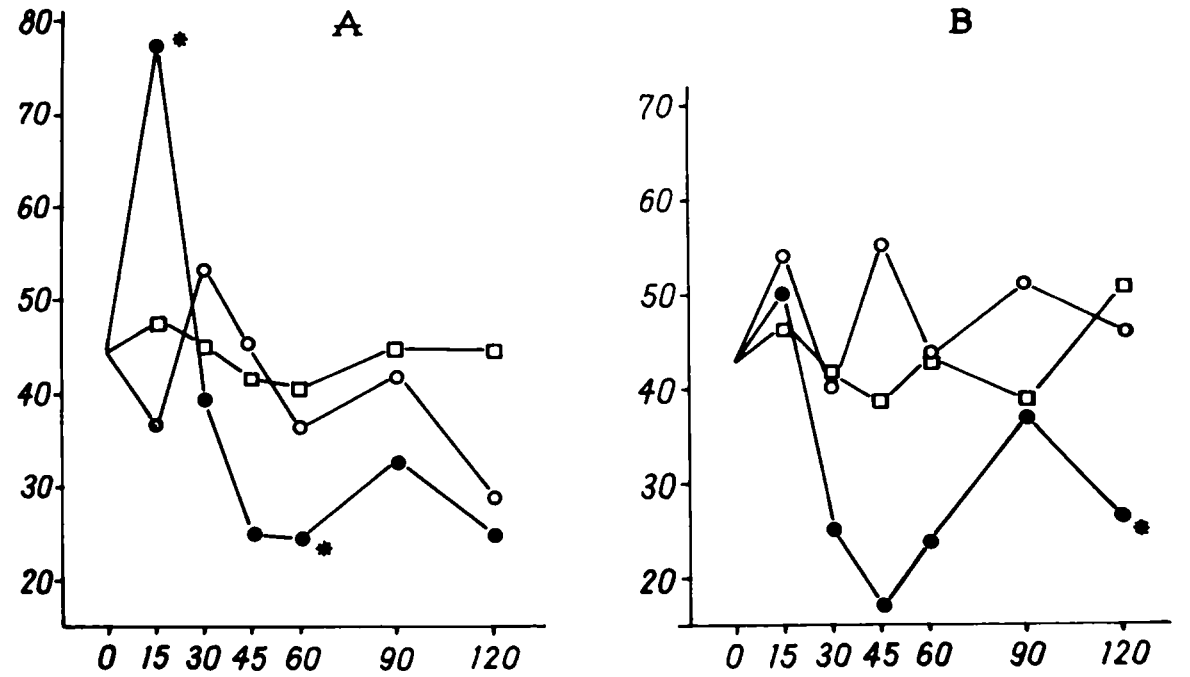

FIG. 3. The effect of raphe lesion on analgesic action of intraventricular SP_{1-11} and PyroGlu in rats. Tail compression test. Panel A: sham-operated animals. Panel B: raphe lesioned animals. Other symbols as for Fig. 2.

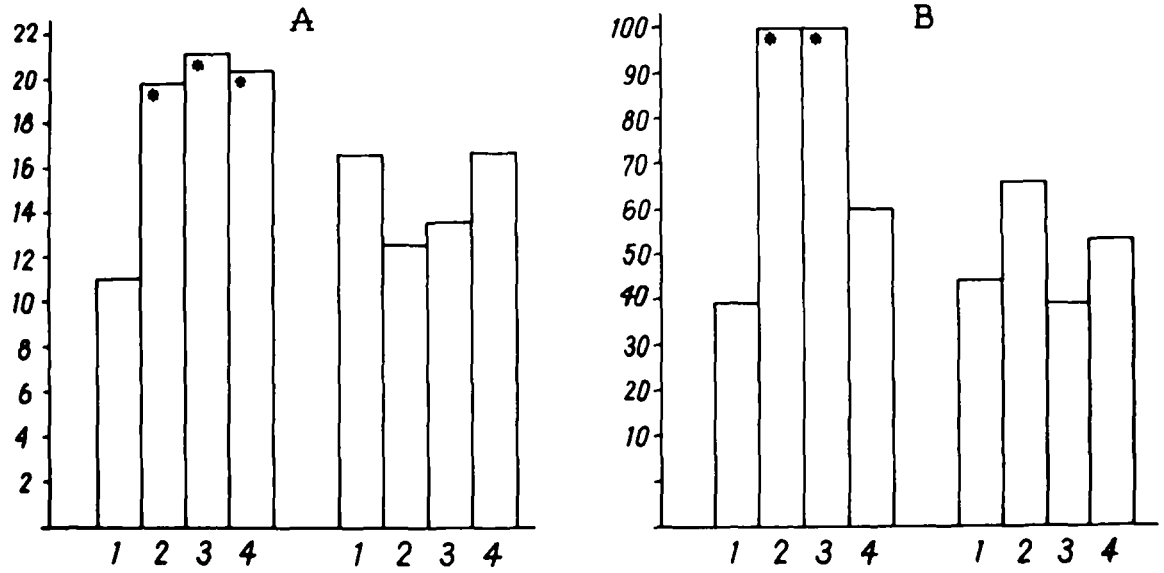

FIG. 4. The effect of PCPA on analgesic activity of SP_{1-11} and PyroGlu in mice (hot plate method). Panel A: latency of hind-paw licking. Panel B: latency of jumping-off. On each panel: left block—saline pretreatment; right block—pretreatment with PCPA. Abscissa: latency in seconds. Ordinates: (1) saline controls; (2) SP_{1-11}, 371 nM/kg; (3) SP_{1-11}, 185.5 nM/kg; (4) PyroGlu, 250 nM/kg. An asterisk denotes significant difference in respect to appropriate saline control.

was not decreased, and after pyroGlu hyperalgesia was not seen.

DISCUSSION

Our results support the previously mentioned evidence for the analgesic activity of SP_{1-11} administered intracerebrally to rats and intraperitoneally in mice. In rats, no reliable data were found concerning the analgesia after systemic administration. In fact, in the pilot series, not included here, intravenous injections of SP_{1-11} to rats in doses up to 50 nM/kg did not cause any detectable change in pain thresholds. It may be thought that in mice this peptide crosses the blood-brain barrier readily, whereas in rats it does not penetrate into the CNS after systemic injection.

The effects of pyroGlu in mice are in accordance with the results published earlier [9]. The lack of analgesic activity in rats after intraventricular injection is then somewhat surprising.

The site of analgesic activity of SP_{1-11} is not clear. Both brain and spinal cord were proposed as a substrate of this effect. The time-course of analgesia, presented above suggests, that at least part of this effect depends on sensitive sites in the brain. However, lack of analgesia after intraventricular pyroGlu in rats seems to be evidence that either this species is insensitive to pyroGlu, or its brain lacks pyroGlu sensitive sites responsible for analgesic activity. The first alternative is unlikely, since pyroGlu was shown to exert some other SP_{1-11}—like effects in rats [5].

It is thought that the most likely explanation of differences between the results obtained in both species is that whereas the parent peptide acts on both brain and spinal sites, pyroGlu acts only on sites localized in the spinal cord. Such sites are probably accessible after systemic injection in mice, but could not be reached after the peptide administered intraventricularly. This may open an interesting speculation on the existence of two populations of SP_{1-11} sensitive receptors, which may differ in their sensitivity to some analogs or fragments of the parent peptide.

The picture arising after 5-HT depletion seems at first somewhat complicated. In mice, PCPA abolished the effects of both peptides, suggesting that serotonergic mechanisms are essential for this type of analgesia. On the other hand, in the rat PCPA did not modify the effects of SP_{1-11}, whereas after lesion of raphe nuclei the analgesia was completely abolished. It should be noted, however, that these two methods of 5-HT depletion are by no means equivalent. The behavioral changes after PCPA differ markedly from those obtained after raphe lesion, and evidence was shown that some features of the so-called "raphe syndrome" are probably not due to the impairment of serotonergic transmission, but to the lesion of some other neurons and/or fibers in the brainstem. In this regard, we did not find any correlation between the degree of 5-HT depletion in lesioned rats and the modification of SP_{1-11} analgesia. Moreover, in some lesioned animals, which did not show any appreciable decrease of 5-HT level, the analgesic activity of SP_{1-11} was abolished, as was seen in those animals with marked 5-HT depletion. These animals with unchanged 5-HT levels were not included in the results, and their number was too small to permit any unequivocal identification of the lesioned structures. The evidence suggests, however, that the lack of SP_{1-11} analgesia in raphe-lesioned rats is probably not due to the depletion of 5-HT but to the interruption of some other neural system which remains to be identified.

The difference between the time-course of analgesia in saline pretreated and sham-lesioned animals, and the presence of hyperalgesia in the latter group is difficult to explain. It was probably not related to any injury of some peptide-sensitive structures by the lesioning electrode, which in our experiments was introduced at approximately a 30° angle to the sagittal plane, in order to spare the central gray, the most likely candidate for the mentioned injury. However, it is possible that the different reactivity of these animals was caused by different time of surgical procedure, which was performed 24 hr before the experiment in saline- and PCPA

pretreated animals, and 4 days before in lesioned and sham-lesioned rats.

The results presented are compatible with differences between the sites of action of SP_{1-11} and its terminal hexapeptide pyroGlu in rats and mice, and the differences between the effect of 5-HT depletion in these species may indicate, that the serotonergic mechanisms play a more important role in pain control in mice than in rats.

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