Non-opioid peptides for analgesia

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Abstract. Amongst the spinal peptide candidates believed to be involved in the mediation of analgesia, only somatostatin fulfills the criterium of a real analgesia substance. Spinal somatostatin specifically blocks the transmission of painful stimuli. Spinal calcitonin may lower the opioid dose requirement in patients with bone metastases but it fails to relieve acute pain. The usefulness of ACTH and CRF for treatment of pain remains to be established. The role of CCK-8, vasopressin and neurotensin is unclear. The contradictory findings on antinociception using simple rodent withdrawal reflex tests (e.g. the tail flick test), or more complex behavioral tests in which supraspinal sensory processing is involved, (e.g. the hot plate test), indicate that these tests are inappropriate when neuropeptides are employed. Furthermore, due to their inability to predict analgesia in humans, they do not fulfill the guidelines proposed by the IASP that animal test procedures have to be for the benefit of humans.

Key words: ACTH, CRF, CCK-8, vasopressin, neurotensin, calcitonin, somatostatin, spinal analgesia
INTRODUCTION

Animal studies indicate that non-opioid peptides are involved in the modulation of sensory processes including pain transmission. Cholecystokinin octapeptide, neurotensin, vasopressin, adrenocorticotrophic hormone (ACTH), corticotropin-releasing factor (CRF), calcitonin and somatostatin are the most promising non-opioid candidates that produce analgesia.

Unfortunately, only few clinical studies give ample evidence for the non-opioid analgesic effect in humans. Contradictory results from rodent test procedures make it difficult to decide whether the one or other non-opioid is an analgesic in man or not.

CHOLECYSTOKININ OCTAPEPTIDE (CCK-8)

Cholecystokinin immunoreactivity is widely distributed within the central nervous system, e.g. in the cerebral cortex, striatum, hippocampus, amygdala and parts of the brain stem. Receptor autoradiographic studies suggest that receptors for CCK-8 are located on axons that may act pre-synaptically to modify the input of sensory information (Innis and Aghajanian 1984). Table I summarizes the effect of CCK-8 on nociceptive thresholds in a variety of common rodent test procedures. Contrasting results were obtained in the rodent tests despite same CCK-8 doses and routes of administration.

<table>
<thead>
<tr>
<th>Rodent Tests</th>
<th>Yes</th>
<th>Reference</th>
<th>Antinociception</th>
<th>No</th>
<th>Reference</th>
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<td>Mouse Hot Plate Jump</td>
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<td>Zetler 1980</td>
<td>50-500 µg/kg sc</td>
<td>Hill et al. 1987</td>
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<td></td>
<td>300 µg/kg sc</td>
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<td></td>
<td>2 µg icv</td>
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<td></td>
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<td>40-3000 µg/kg sc</td>
<td>Hill et al. 1987</td>
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<td>15-60 ng ith</td>
<td>Hong and Takemori 1989</td>
<td>120-1920 ng itch</td>
<td>Hong and Takemori 1989</td>
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<tr>
<td></td>
<td>15-60 icv</td>
<td>Takemori 1989</td>
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<td></td>
<td>750 µg/kg sc</td>
<td>Zetler 1980</td>
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<tr>
<td>Phenylchino</td>
<td>4.5 µg/kg icv</td>
<td>Barbaz et al. 1986</td>
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<td></td>
<td>3 µg/kg sc</td>
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<td></td>
<td>2,000 µg/kg sc</td>
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<td>Acetylcholin</td>
<td>1,100-10,000 µg/kg sc</td>
<td>Hill et al. 1987</td>
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and even when the same test procedure was used, e.g. the mouse hot plate test (Hill et al. 1987, Zetler 1980) or the rat paw pressure test (Hill et al. 1987). Baber et al. (1989) postulated that "pharmacological" doses of CCK-8 in contrast to small CCK-8 doses produce analgesia. However, part of the CCK-8 doses that produced antinociception in the rat tail flick, rat paw pressure and mouse writhing tests were lower (Hong and Takemori 1989; Jurna and Zetler 1981, Pittaway et al. 1987) than the doses that produced hyperalgesia or were without effect (Table I). The fact that the results obtained in most studies could not be reproduced by other groups left the question unanswered as to whether CCK-8 is an analgesic substance or not.

Rodent test procedure (the rat hot plate and tail flick tests) give evidence that the CCK-8 antagonist proglumide potentiates opioid analgesia, even when administered systemically (Barbaz et al. 1985). It was, however, also suggested that CCK-8 might antagonize opioid analgesia (Katsuura and Itoh 1985, Watkins et al. 1985). Three clinical investigations produced further contradictory findings on the effect of proglumide on morphine analgesia in humans. Whereas 50 pg intravenous proglumide potentiated the analgesic effect of morphine after inducing experimental pain (Price et al. 1985) or following surgical removal of all four third molar teeth (Lavigne et al. 1989), 0.5 μg, 100 μg and 50 mg intravenous proglumide have proved to be ineffective in potentiating morphine analgesia for the alleviation of pain after abdominal operations (Lehmann et al. 1989). It therefore seems unlikely that the neuromodulatory role of CCK-8 in antinociception (Wiesenfeld-Hallin and Duranti 1987) is of clinical relevance.

NEUROTENSIN

The tridecapeptide neurotensin is mainly distributed throughout the periaqueductal gray, substantia gelatinosa and locus coeruleus (Uhl et al. 1979). The anatomical localization of neurotensin receptors in the deeper inner segment of the spinal cord also indicate their involvement in modulating sensory functions (Faull et al. 1989). A naloxon-irreversible analgesia was achieved in the rat hot plate and tail flick tests following administration of neurotensin into the periaqueductal grey. Electrophysiological studies revealed that the analgesic effect was elicited by excitation of neurons that activate descending inhibitory nerve fibres to dorsal horn neurons involved in the mediation of pain (Behbehani and Pert 1984). Similarly, direct microinjection of neurotensin into the central nucleus of the amygdala produced a significant increase in the antinociceptive threshold when using the rat hot plate test. Lesions of the stria terminalis totally abolished this antinociceptive effect (Kalivas et al. 1982).

However, equal intra-cerebroventricular doses of neurotensin produced antinociception in the mouse hot plate test (Osbahr et al. 1981), but not in the rat hot plate test (Kalivas et al. 1982). Furthermore, the antinociceptive response in the rat tail flick test with equivalent intra-cerebroventricular doses of neurotensin differed (Clineschmidt et al. 1979, Pazos et al. 1984) (Table I). The rodent test procedures did, therefore, not clearly demonstrate the neurotensin antinociceptive effect.

VASOPRESSIN

The pituitary polypeptide hormone vasopressin is present in the brain (Sofroniew 1980) and cerebrospinal fluid (Jenkins et al. 1980). The release of vasopressin in man is controlled by endogenous opioids (Rossier et al. 1979). This may suggest a linkage between vasopressin and pain. The contradictory results when equal doses of intrathecal vasopressin were employed in the rat tail flick test (Millan et al. 1980, Thurston et al. 1988) make it difficult to predict whether intrathecal vasopressin is an analgesic in humans (Table II).

ACTH

The pituitary peptide ACTH acts like an agonist-antagonists at central opioid receptors. Opioid induced analgesia in the rat hot plate and tail-flick
### TABLE II

Effects of neurotensin and vasopressin in common rodent test procedures, the dosages employed, routes of administration (ith, intrathecally; icv, intra-ventricularly), antinociceptive findings and references

<table>
<thead>
<tr>
<th>Rodent Tests</th>
<th>Yes</th>
<th>Reference</th>
<th>Antinociception</th>
<th>No</th>
<th>Reference</th>
</tr>
</thead>
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<td>Neurotensin</td>
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<td>Osbarh et al. 1981</td>
<td></td>
<td>2.5 µg icv</td>
<td>Kalivas et al. 1982</td>
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<td>Rat Hot Plate</td>
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<tr>
<td>Rat Tail Flick</td>
<td>5.4 µg icv</td>
<td>Pazos et al. 1984</td>
<td></td>
<td>2.4 µg icv</td>
<td>Clineschmidt et al. 1979</td>
</tr>
<tr>
<td>Vasopressin</td>
<td></td>
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<tr>
<td>Rat Tail Flick</td>
<td>2.5 and 25 ng itch</td>
<td></td>
<td>0.1-20 µg itch</td>
<td>Millan et al. 1984</td>
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</table>

Intracerebroventricular administration of up to 30 µg CRF has failed to produce analgesia in the hot plate (Sherman and Kalin 1986) and the tail flick (Ayesta and Nikolarakis 1989) tests.

However, intravenous CRF (12.5 µg/kg) increased the latency in the rat hot plate test (Hargraeves et al. 1987). Moreover, the analgesic effect of CRF was demonstrated in 14 patients having bilaterally symmetrical impacted third molars. At each procedure an upper and lower third molar on one side was extracted under local anesthesia consisting of 2% mepivacaine. Sixty minutes following surgery, subjects received intravenously either 1 µg/kg human CRF or placebo. At a second operation approximately 2 weeks later the alternative treatment was administered. Mean pain scores were significantly lower at 90 and 120 minutes in the patients having received intravenous CRF (Hargraeves et al. 1987). It was suggested that an increase in β-endorphine release may account for the analgesic CRF effect. Moreover, a pain-relieving effect via glucocorticoids may also be possible since CRF stimulation of the pituitary-adrenal axis leads to increased levels of antiinflammatory glucocorticoids (Hargraeves et al. 1989).

### CRF

CRH is widely distributed in hypothalamic and extrahypothalamic brain areas known to be involved in the initial processing of nociceptive signals (Schipper et al. 1983, Skofitsch et al. 1985). A physiological role of ACTH in antinociception was recently questioned. Administration of dexamethasone prior to the footshock test in rats did not influence the stress antinociceptive threshold although dexamethasone is known to inhibit ACTH release (Millan et al. 1984). Therefore it is still unknown whether ACTH is of value in treatment of clinical pain.

### CALCITONIN

The 32 amino acid peptide calcitonin produced by the thyroid glands derives from the same gene as calcitonin gene-related peptide (CGRP), a central...
peptide possibly involved in the mediation of analgesia (Bates et al. 1984). The assumption that calcitonin may interact with CGRP central receptor sites was the reason to investigate salmon calcitonin in those rodent test procedures believed to predict analgesia in man. Intrathecal salmon calcitonin produced a dose-dependent, reversible increase in the latency of licking a hindpaw in the rat hot plate test but failed to attenuate the vocalization threshold. Wiesenfeld-Hallin and Persson (1984) suggested therefore that salmon calcitonin has no antinociceptive effect but rather a reversible blocking effect on the motoric system. In humans, epidural salmon calcitonin was ineffective in alleviating acute postoperative pain, although there was clearly a pain-relieving effect of epidural or systemic salmon calcitonin in cancer patients with bone metastases (Allan 1983, Chrubasik et al. 1986, Fiore et al. 1983, Fraiolii et al. 1982, Schiraldi et al. 1987). Motoric disturbances were not observed in patients although their occurrence had been predicted in the rodent test procedures.

Because calcitonin fails to block transmission of acute pain stimuli, this peptide is per definition not an analgesic substance. Calcitonin may be classified as an analgesiapotentiating substance in cancer pain patients suffering from bone metastases.

**SOMATOSTATIN**

Cell populations containing the tetradecapeptide somatostatin are particularly concentrated in certain areas of the brain, e.g. the midbrain central grey, amygdala and medulla (Johansson et al. 1984). In the spinal cord the highest concentrations of somatostatin were found in the Rexed Laminae II and III in the region of the grey matter of the dorsal horn (Seybold and Elde 1980). This area is known to be involved in the transmission of somatosensory information, particularly with nociceptive afferent impulses.

Preliminary experiments in rats, cats and mice did not indicate any analgesic effect of intrathecal somatostatin below the neurotoxic dose range (Gaumann and Yaksh 1988, Gaumann et al. 1989). Only recently it has been found that antinociception, motor effects and neurotoxicity of spinal (intrathecal, epidural) somatostatin can be distinguished dependent on the dose administered into the intrathecal or epidural space (Mollenholt et al. 1990). Table III summarizes the somatostatin therapeutic and toxic intrathecal and epidural dose range.

The analgesic effect of epidural somatostatin has been demonstrated in humans. A dermatomal distribution of analgesia similar to that seen with epidural local anesthetics occurred following epidural administration of 1 mg somatostatin. In contrast to local anesthetics, the segmental dermatome analgesic limits, determined by pin prick stimuli, were independent of the injection volume and could be maintained under continuous low dose, epidural infusion of somatostatin (Fig. 1). A subsequently higher epidural infusion dose (1 mg)

<table>
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<th>Therapeutic</th>
<th>Reference</th>
<th>Toxic</th>
<th>Reference</th>
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<tr>
<td>a) Rats</td>
<td>&lt; 50 µg/kg</td>
<td>Mollenholt et al. 1990</td>
<td>&gt; 50 µg/kg</td>
<td>Mollenholt et al. 1990</td>
</tr>
<tr>
<td>Mice</td>
<td>&lt; 5 µg/kg</td>
<td>Chrubasik et al. 1984</td>
<td>&gt; 150 µg/kg</td>
<td>Gaumann and Yaksh 1988</td>
</tr>
<tr>
<td>Cats</td>
<td></td>
<td></td>
<td>&gt; 150 µg/kg</td>
<td>Gaumann et al. 1989</td>
</tr>
<tr>
<td>Humans</td>
<td></td>
<td></td>
<td>&gt; 150 µg/kg</td>
<td>Gaumann et al. 1989</td>
</tr>
<tr>
<td>b) Rats</td>
<td>&lt; 800 µg/kg</td>
<td>Mollenholt et al. 1990</td>
<td>&gt; 800 µg/kg</td>
<td>Mollenholt et al. 1990</td>
</tr>
<tr>
<td>Dogs</td>
<td>&lt; 100 µg/kg</td>
<td>Chrubasik et al. 1984</td>
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<tr>
<td>Humans</td>
<td>&lt; 20 µg/kg</td>
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hourly following the initial bolus dose (0.5 mg) even produced a total body negative pin-prick response (Hugler et al. 1987). Spinal (intrathecal, epidural) somatostatin has been used successfully to alleviate acute and chronic pain (Chrubasik et al. 1984, Chrubasik et al. 1985). It needs to be established, however, why in some patients somatostatin did not produce sufficient pain relief (Chrubasik 1988, Hugler et al. 1987).

Control of ventilation whilst breathing room air and during carbon dioxide stimulation revealed that epidural somatostatin does not influence the respiratory variables (Fig. 2). Moreover, the postoperative analgesic somatostatin effect was not reversible by naloxone (Chrubasik et al. 1985). Somatostatin must, therefore, act via specific central receptor sites.

A recent pilot study reported that the somatostatin analogue octreotide is also a potent analgesic. Patients suffering from intractable cancer pain resistant to opioids experienced a considerable reduction in pain over periods up to 3 months (Penn et al. 1992).

NON-OPIOID PEPTIDES AND RODENT TEST PROCEDURES

The contradictory findings on antinociception when using non-opioids, e.g. CCK-8, neurotensin and vasopressin, and rodent test procedures indicate that these tests are inappropriate for the evaluation of the non-opioid analgesic effectiveness.

There is no doubt that the results on nociception may also vary with the intensity of the stimuli employed. Intrathecally administered kappa opioid receptor agonists, for example, have failed to produce analgesia against high intensity heat stimuli, but have been very effective against low intensity heat stimuli (Millan 1989; Parsons and Headley 1989). This suggests that various stimuli need also to be considered whilst testing spinal non-opioid peptides in rodent test procedures.

Without clear prescriptions the results of rodent test procedures are without any value and do not fulfill the guidelines proposed by the IASP that animal test procedures have to be for the benefit of humans.

REFERENCES


Paper presented at the 1st International Congress of the Polish Neuroscience Society; Session: Pain: analgesia and neuropeptides