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BRAIN RESEARCH

Research Report

Arthritis-induced increase in cholecystokinin release in the rat anterior cingulate cortex is reversed by diclofenac

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ARTICLE INFO

Article history:
Accepted 5 December 2006
Available online 16 January 2007

Keywords:
Anterior cingulate cortex
Cholecystokinin
Pain
Microdialysis
Rat
Morphine
Diclofenac

ABSTRACT

Given a hypothesised role for CCK in the anterior cingulate cortex (ACC) for the sensation of pain, the aim of the present study was to investigate whether the increased CCK release could be affected by two different analgesic drugs, morphine and the non-selective cyclooxygenase inhibitor diclofenac. Since opioids stimulate CCK release in other CNS regions we have also studied the effect of morphine by itself on the CCK-LI release in the ACC of non-arthritic rats. Three to seven hours after intraarticular carrageenan injection, at the time when the animals are known to show pain-related behaviour, in vivo microdialysis in awake rats revealed increased CCK-LI release in the ACC. The CCK-LI release was significantly attenuated by diclofenac (25 mg/kg i.m.), but not by morphine (10 mg/kg s.c.). Neither diclofenac (25 mg/kg i.m.) nor morphine (5 or 10 mg/kg s.c.) affected the CCK-LI release in the ACC in non-arthritic rats. The results obtained with diclofenac indicate that prostaglandins contribute to the increased CCK-LI release in the ACC during monoarthritis. However, the lack of effect of morphine suggests that the CCK release in the ACC is not directly related to the sensation of pain. Further on, the failure of morphine to affect the extracellular level of CCK-LI in the ACC in control animals as well as in animals with carrageenan-induced monoarthritis is in contrast to previous studies on the frontal cortex or the dorsal horn of the spinal cord, in which similar doses of morphine stimulate CCK release. Thus, compared to these regions, CCK release may be differently regulated in the ACC.

1. Introduction

The involvement of the neuropeptide CCK in pain modulation is well established especially regarding anti-opioid mechanisms (Cesselin, 1995; Hebb et al., 2005; Mollereau et al., 2005). It has for instance been shown that CCK agonists attenuate (Faris et al., 1983; Itoh et al., 1982; Wang et al., 1990), whereas CCK antagonists enhance the anti-nociceptive effect of exogenous opioids such as morphine (Dourish et al., 1990; Watkins et al., 1984). CCK antagonists can also enhance the analgesic effect of experimentally induced increase of endogenous opioids in rats (Han et al.,

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Abbreviations: CCK-LI, cholecystokinin-like immunoreactivity; ACC, anterior cingulate cortex; NSAID, non-steroid anti-inflammatory drug; RVM, rostral ventromedial medulla; RIA, radioimmunoassay; PG, prostaglandin; COX, cyclooxygenase

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doi:10.1016/j.brainres.2006.12.006

1986; Valverde et al., 1994; Watkins et al., 1985) and humans (Benedetti, 1996). Whether CCK also has pronociceptive effects by itself has been a subject of debate, but there is evidence that CCK directly facilitates nociception in the rostral ventromedial medulla (RVM) (Heinricher and Neubert, 2004). Particularly at spinal level, interactions between CCK and opioids have been extensively studied (Stanfa and Dickenson, 1995; Wiesenfeld-Hallin and Xu, 2001). A possible pain-regulating function for CCK on higher CNS levels is, on the other hand, less well explored.

The mechanisms regulating the release of CCK are far from clear. Different subtypes of opioid receptor agonist seem to affect the CCK release differentially in a complex way although the delta-opioid receptor seems to be of special importance (see Wiesenfeld-Hallin and Xu, 2001). For example, systemic and local administration of morphine increases the spinal release of CCK-LI and this increase is mediated by delta-opioid receptors (de Araujo Lucas et al., 1998; Gustafsson et al., 2001). Data from the frontal cortex show that acute or subchronic administration of morphine increases the release of CCK-LI and, as observed in the spinal cord, this effect could be blocked by delta-opioid antagonists (Becker et al., 1999, 2000).

In addition to pain modulation, central CCK has also been implicated in anxiety and stress (Bradwejn and Koszycki, 2001; Rotzinger and Vaccarino, 2003; Van Megen et al., 1996). For instance, administration of CCK-B receptor agonists induces anxiety in humans (Koszycki et al., 1998; Radu et al., 2003) and increases anxiety-like behaviour in animals (Wang et al., 2005), whereas CCK-B receptor antagonists or antisense appears to have anxiolytic effects, at least in rodents (Herranz, 2003; Tsutsumi et al., 2001; Woodruff and Hughes, 1991). Recently, it was reported that rats subjected to chronic stress in a social-defeat model, as compared to control animals, showed significantly more pain-related behaviour which was accompanied by a significant increase in CCK-LI release in the frontal cortex (Andre et al., 2005). This indicates a close interaction between nociceptive and emotional functions with a possible involvement of CCK in this brain region.

There is a growing body of evidence from both human (Apkarian et al., 2005; Casey, 1999; Villemure and Bushnell, 2002) and animal (Treede et al., 1999; Willis and Westlund, 1997) studies that another cortical area, the anterior cingulate cortex (ACC), plays an important role in pain processing. In the rat, nociceptive neurons with body receptive fields and bilateral nociceptor innervations have been found in the ACC (Kuo and Yen, 2005; Yamamura et al., 1996). In addition, there are interconnections of the ACC with other brain regions involved in pain modulation such as the periaqueductal grey, thalamus and somatosensory cortices (Schnitzler and Ploner, 2000; Vogt, 2005). Moreover, prolonged noxious stimulation (Liu et al., 1998) as well as pain-related aversion (Lei et al., 2004) induces expression of the immediate early gene c-*fos* in the ACC, suggesting that this brain region is activated during pain. Studies in humans with functional imaging techniques (Coghill et al., 1999; Rainville et al., 1997) together with behavioural studies in rodents (Johansen et al., 2001; Kung et al., 2003) and primates (Koyama et al., 2001) indicate that the ACC is involved in the affective, rather than the sensory discriminative, component of pain (Price, 2000; Rainville, 2002). Indeed, it was recently reported that activation of the ACC in humans was directly correlated to pain-related anxiety and fear (Ochsner et al., 2006). Interestingly, CCK is especially abundant in the ACC in both humans (Emson et al., 1982; Savasta et al., 1990) and rodents (Beinfeld et al., 1981; Hökfelt et al., 1991), and there is a high density of CCK-B receptors in this region (Pelaprat et al., 1988; Suzuki et al., 1993; Woodruff et al., 1991). Thus, it is conceivable that CCK, as in other CNS regions, plays a pain-regulating role also in the ACC.

We have previously demonstrated that the CCK-LI release is increased in the ACC during acute carrageenan-induced monoarthritis (Erel et al., 2004). An increased CCK-LI release in this brain region has also been reported following peripheral axotomy of the sciatic nerve, a model of phantom limb pain (Gustafsson et al., 2000). In order to test whether CCK in the ACC is involved in the modulation of pain, specifically the affective component, we have investigated whether the increased CCK release observed in arthritic rats could be affected by two different analgesic drugs, morphine and the NSAID diclofenac. Morphine is known to have effects on both the nociceptive and the affective component of pain, whereas NSAIDs are antinociceptive but are virtually devoid of effect on the affective component. Here, we have compared the effects of these two analgesic drugs on the arthritis-induced increase in CCK-LI release in the ACC using in vivo microdialysis in awake, freely moving rats. Since morphine has previously been shown to dose-dependently increase the release of CCK-LI by itself in other CNS regions (Becker et al., 1999; Gustafsson et al., 2000) and since the ACC has one of the highest densities of opioid receptors in the CNS (Sprenger et al., 2005; Zubieta et al., 2001), we also studied the effect of morphine alone on the CCK-LI release in the ACC of non-arthritic rats.

2. Results

2.1. *Effect of morphine or diclofenac on arthritis-induced CCK-LI in the ACC*

In rats injected with carrageenan into the tibio-tarsal joint the CCK-LI release was increased at all time points during dialysis collection as compared to control rats (Fig. 1A). The mean release (±SEM) of CCK-LI measured between 3.0 and 7.0 h after injection of carrageenan, i.e. during the time period when maximal pain-related behaviour is observed (Erel et al., 2004), was significantly increased in animals with carrageenan-induced arthritis (3.9±0.2 pM) compared to controls (2.3±0.1 pM; Fig. 1B). Administration of 10 mg/kg of morphine (s.c.) prior to carrageenan injection did not affect the carrageenan-induced CCK-LI release (Fig. 1A). Thus, the mean (±SEM) release of CCK-LI at 3.0–7.0 h after carrageenan injection was not significantly different in animals treated with morphine (3.74±0.2 pM), compared to rats treated with saline (3.9±0.2 pM; Fig. 1B). In contrast, pre-treatment with 25 mg/kg of diclofenac (i.m.) counteracted the carrageenan-induced CCK-LI increase (Fig. 1A). Thus, the mean (±SEM) CCK-LI release during 3.0–7.0 h after carrageenan-induced monoarthritis was