Evidence for time dependence of morphine effects on cholecystokinin release from rat periaqueductal grey

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The peptide neurotransmitter cholecystokinin (CCK) is widely distributed in the central nervous system (CNS) and is thought to be involved in a variety of centrally mediated behaviours. Of particular interest is the suggestion that opioid and CCK systems interact in an antagonistic manner to influence behaviours including antinociception (Faris, 1985). Neurochemical studies show a similar interaction. Opiates are reported to inhibit CCK release from hypothalamic slices in vitro (Micevych et al., 1982, 1984) and CCK levels in hypothalamus are elevated after morphine injection (Faris et al., 1986). Similarly we have shown that the release of CCK from slices of a brain region involved in pain control, the periaqueductal grey (PAG) is modulated by opiates (Rattray & de Belleroche, 1986a, 1987). Our results imply that opiates have an inhibitory effect on CCK release from PAG which, in this tissue, is masked unless the tissue is naloxone pretreated. In the present study, the time dependence of the effects of morphine on PAG CCK release was studied.

Male CFY rats (250-350 g) were killed and their brains removed. The PAG were dissected out and chopped into slices of 0.3 mm thickness. Slices were incubated at 37°C in Krebs/bicarbonate medium containing 0.5 mM-bacitracin and gassed with 95% O2/5% CO2. After a preincubation period of 15-60 min in the presence or absence of 10^-6 m

marginally lowered only after 2 h ischaemia. This change in permeability of the plasma membrane which set in after the point of irreversible cellular alteration.

Abbreviations used: CCK, cholecystokinin; PAG, periaqueductal grey.

Fig. 1. Effect of morphine preincubation on K+ evoked CCK release

PAG slices were incubated in the presence of 10^-6 m

morphone (●) or no drug as control (○) for 15-60 min before incubation, without drug, for a 10 min basal period followed by 5 min in 34 mM-K+. Results represented as means ± S.E.M. K+ evoked CCK release values from morphine pretreated slices were compared to controls incubated for the same time period using a paired t-test.
extract low molecular mass forms of CCK. The methanolic extracts were dried under nitrogen and stored at –20°C until redissolved for assay. Tissue and released levels of CCK were measured by radioimmunoassay. The antibody Ab2717 (a gift of Professor J. Rehfeld) was used at a dilution of 1:600000. Synthetic human gastric (1-17) (Cambridge Research Biochemicals) was used as a tracer. K⁺-evoked CCK release was calculated as the percentage of the total CCK-LI (supernatant + tissue) released per minute of incubation in 34 mM-K⁺ minus the percentage CCK released per min during the basal incubation period. Drug-treated release values were compared to their own controls using a paired t-test.

When PAG slices were exposed to morphine (10⁻⁶ m) for 30–60 min before incubation without drugs, K⁺-evoked release of CCK was enhanced (Fig. 1). The greatest effect (71 ± 36% increase, n = 7, P < 0.05) was observed after 30 min morphine preincubation. At 45 or 60 min preincubation, morphine-induced increases in CCK release were apparent, but not significant (P < 0.10).

The effect of prolonged morphine treatment on PAG CCK release is strikingly different from the morphine-induced inhibition of PAG CCK release, observed over a shorter exposure time (Rattray & de Belleroche, 1987). The increases observed on PAG CCK release in vitro compare with observed increases in CCK octapeptide and CCK mRNA levels which occur in PAG after morphine injection (Rattray & de Belleroche, 1986b). This suggests that CCK synthesis and release are coupled and that morphine may exert these delayed effects by a post-receptor action modulating protein synthesis.

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Towards understanding the role of the Na–Ca exchanger in cardiovascular activities: utilization of harmaline

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The sarcolemmal Na–Ca exchange system is an important component of the mechanism whereby changes in intracellular Na concentration are translated into changes in developed tension in mammalian cardiac tissue. It might have an important role in bringing about the final physiological response, since intracellular organelles, especially the sarcoplasmic reticulum, participate directly in regulating the intracellular Ca concentration (Chapmann, 1983; Chapmann & Rodrigo, 1983; Reeves, 1984). It is obvious, therefore, that a direct correlation between sarcolemmal Na-Ca exchange activity and physiological processes is somewhat difficult to obtain. In this respect, the utilization of a cardioactive agent, which also inhibits the Na–Ca exchanger, would be an advantage. Agents such as quinacrine and amiloride (Sutko et al., 1983; Slaughter et al., 1984) and verapamil (Erdreich et al., 1983), were found to inhibit the Na–Ca exchanger. However, none of these agents seem to be an appropriate tool, since the effect did not parallel their response in vivo. Harmaline, which was found to have a series of cardiovascular actions (Zetter, 1974; Carpenter & Narvarte, 1975; Carpenter & Diaz, 1977; Aarons et al., 1977; Carpenter, 1980), was also found to inhibit the Na–Ca exchange mechanism in ideal smooth muscles (Suleiman, 1985; Suleiman & Hider, 1985) and in cardiac sarcolemmal vesicles (Suleiman & Reeves, 1987). In the case of the latter, harmaline was found to be a competitive inhibitor with respect to Ca (Kᵢ = 2.5 ± 10⁻⁴ m). Furthermore, in preparations both in vitro and in vivo, the inhibitory potency demonstrated by harmaline was similar. This finding is significant and provides a tool necessary for the elucidation of the contribution of the Na–Ca exchanger in the physiological activities of cardiac muscle.


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