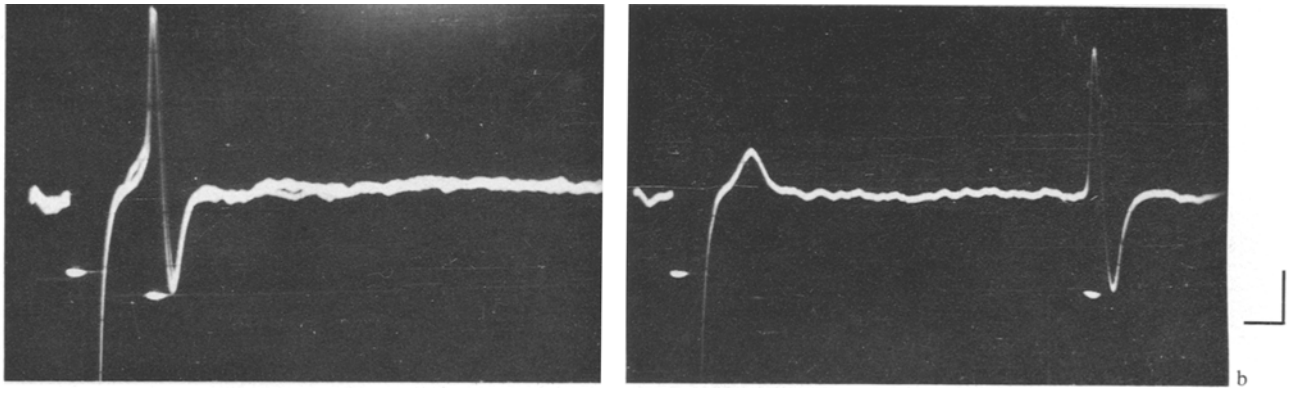

Fig. 2. Antidromic activation and cancellation by a spontaneous spike in a neuron in the SN, after stimulation of the n.r.g.c. a) Antidromic spike showing constant latency (3 superimposed sweeps). b) Cancellation by a spontaneous spike which triggered both the stimulator and the scope sweep. Only the SD component is present as the stimulus failed to evoke a full spike. Dots indicate occurrence of stimulus artefact and of spikes. Calibration: 5 msec; 100 µV.

excited 2 units with similar latencies. On 3 occasions while stimulating from the n.r.g.c., neurons showing antidromic activation (constant latency and ability to follow frequencies up to 500 Hz) were detected, and cancellation by a spontaneous spike was obtained (figure 2).

The present results provide electrophysiological evidence of a reciprocal link between the SN and the medullary RF, the connection between the SN and the RF being ipsi and contralateral. Although fibres en passage could have been activated, the finding of antidromically activated neurons, demonstrates a direct link of a monosynaptic nature. At present it is not known whether there are separate projections to both sides of the brain or whether there is a branching of the same axons which can cross the midline to innervate the contralateral side. This pathway could be the output from the SN to the spinal cord, through reticulospinal neurons, as has already been suggested[2,11]. It is interesting to note that both ipsi and contralateral effects have been reported after SN stimulation[2,12], albeit of a different nature. More recently, alterations in EEG activation from the RF have been described after making lesions in the SN[13]. It is still premature to asign a function to the reticulo-nigral connection found in this study, but SN units show responses to peripheral stimulation not mediated by diencephalic structures[14], and these effects could be relayed via the RF. The n.r.g.c. is known to receive afferents from peripheral receptors[15], and it may be that feedback information from several receptors including muscle afferents is being conveyed to the SN as part of its regulatory role in motor control.

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Methionine enkephalin inhibits the bursting activity of the Br-type neuron in *Helix pomatia* L.

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Summary. The present study demonstrates that methionine enkephalin can inhibit the normal bursting activity pattern of the RPal or Br-type neuron and this inhibition can be blocked by prior treatment with naloxone, the selective opiate antagonist. The study demonstrates indirectly the presence of opiate-like receptors in *Helix pomatia.*

Recent reports on the pharmacological effects of methionine enkephalin and morphine on bivalves[3,4] have strongly suggested the presence of opiate receptors in invertebrates. Specific binding analysis using ^{125}I-labeled FK 33824 on *Mytilus edulis* pedal ganglia demonstrated the existence of stereo-specific opiate receptors[5]. A more recent study has localized enkephalin and B-endorphin in the earthworm[6]. As a result of the above, the present study examined the effect of methionine enkephalin on the bursting pattern of the Br-type neuron, since this neuron responds to dopamine and this effect is antagonized by haloperidol and naloxone[7].

Materials and methods. Specimens of *Helix pomatia* employed for this study were collected locally in the woods surrounding the Biological Research Institute at Tihany. The preparation of the tissue, cellular identification and recording technique have been described previously[7,8].

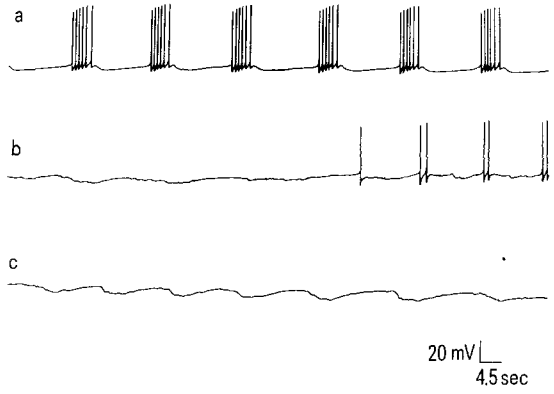

Fig. 1. Effect of methionine enkephalin (1 µg/ml) on the bursting activity of the Br-type neuron. a, control activity; b, immediately after agent application; c, 1 min after agent application – only slow wave activity observed.

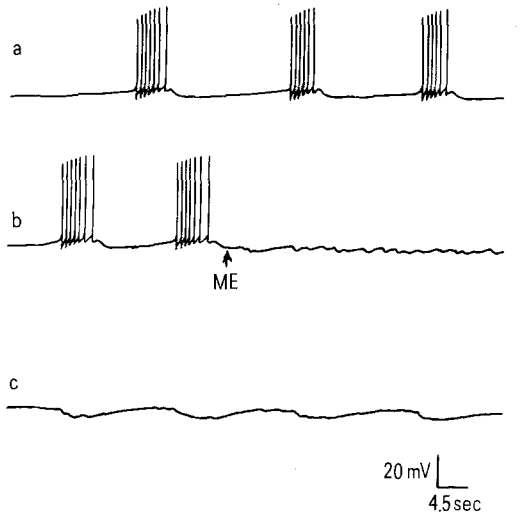

Fig. 2. Effect of a lower dose of methionine enkephalin (ME) (0.1 µg/ml) on the Br-type neuron. a, control activity; b, agent application; c, 2.5 min after application.

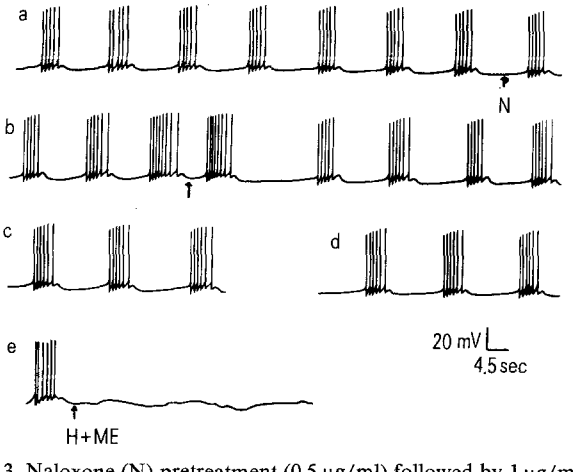

Fig. 3. Naloxone (N) pretreatment (0.5 µg/ml) followed by 1 µg/ml methionine enkephalin (ME). a, control activity and naloxone application; b, methionine enkephalin application; c, 1 min later; d, 2 min later; e, effect of 1 µg/ml of methionine enkephalin and 10 µg/ml haloperidol (H) on the bursting activity of the Br-type neuron.

Results and observations. Control preparations of the Br-type neuron, and preparations in which only vehicle was applied to the cell surface, exhibited bursting activity in a very regular and predictable manner[7,8] (figures 1 and 2). Methionine enkephalin application (0.01–1 µg/ml) to this neuron caused inhibition of the bursting activity. After treatment the cells exhibited no spike generation; however, the slow waves underlying the bursting activity were still observed. Immediately after applying the lower dose of methionine enkephalin only EPSP were observed, with spikes. This inhibitory effect lasted for about 4 min after which normal activity reappeared. The inhibitory effect of this pentapeptide is somewhat different from that which occurs after dopamine treatment[7]. Dopamine inhibition involves a progressive increase in the interburst intervals until no activity can be observed and this inhibition lasts for about 1–2 min. This is then followed by periods of spike activity.

The potent opiate receptor blocker naloxone[9] (0.5 µg/ml), when administered to the Br-type neuron, causes no visible alteration of the cell's activity[7]. Pretreating the cell with naloxone blocked the effects of the pentapeptide. However, there was a slight initial increase in the interburst interval which was rapidly followed by normal bursting (figure 3). In the present study haloperidol did not in any way antagonize the effects of methionine enkephalin (figure 3, e). Dopamine effects on this neuron are counteracted by both haloperidol and naloxone[7] whereas enkephalin effects are just counteracted by naloxone. The differences observed between the 2 types of inhibition produced by the respective agents and the pharmacological blockade may be explained by the existence of 2 separate receptors, or the same receptor having allosteric properties capable of eliciting the same activity by different mechanisms. In addition, the data may be interpreted as involving activation of pre- and post-synaptic receptors. Application of drugs to the cell could result in multiple receptor interactions. Some of the interactions may be direct while others are indirect. At present, the possible theories explaining the above are too numerous to mention and will be the subject of future investigations.

Microelectrophoretic application of methionine enkephalin to single neurons in the mammalian CNS results in a decrease in the spontaneous firing rate and this effect is blocked or reversed by naloxone[10]. This same phenomenon has now been demonstrated in *H. pomatia.* In other reports involving methionine enkephalin and dopamine metabolism in invertebrates a link is believed to exist between the pentapeptides and dopamine metabolism[3,4].

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