## Naloxone shortens ejaculation latency in male rats

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*Summary.* Naloxone, a specific inhibitor of opioid receptors, lowers ejaculation threshold in the male rat coupled with receptive females.

We have reported that the intraventricular injection of D-Ala[2]-Met-enkephalinamide (DALA), a synthetic analog of met-enkephaline, resistant to enzymatic degradation, suppresses the copulatory behaviour in sexually vigorous male rats in doses in which it does not influence motor activity or produce other overt behavioural changes[3].

Similar results have been obtained by Meyerson and Terenius, with intraventricularly injected $\beta$-endorphin[4].The inhibitory effect of the above peptides seems to be due to the stimulation of opioid receptors, since it is prevented by naloxone[3] or naltrexone[4], specific opioid receptor antagonists. Moreover, we have observed that naloxone induces copulatory behaviour in sexually inactive male rats paired with receptive females[5].

These findings suggest that endogenous endorphins, a generic name for peptides with morphine-like activity comprising enkephalins and endorphins sensu strictu, may play an important role in the regulation of copulatory behaviour.

In an attempt further to clarify the possible role of endorphins in the regulation of sexual behaviour, we studied the effect of naloxone on the copulatory pattern of sexually active rats. This study shows that rats treated with naloxone achieve the 1st ejaculation sooner and after fewer intromissions than control rats.

*Methods.* Male Sprague-Dawley rats (purchased from Charles River, Como, Italy) were used. At the beginning of the experiments the animals were approximately 100 days old and weighed 280–300 g. The animals were housed individually in $20 \times 40$ cm cages starting at least 1 week before the beginning of the experimental period, under a reversed light-dark cycle (with light from 23.00 to 11.00 h), at 24 °C and fed ad libitum. 40 vigorous copulators were selected for the present study after 4 mating tests with receptive females, as previously described[6]. The females used as copulatory partners were ovariectomized Sprague-Dawley rats brought into heat with estradiol and progesterone[7]. Mating tests were carried out during the dark phase of the cycle, from 17.00 to 19.00 h in dim red light.

A female was introduced into the male's own cage and the test was terminated after 30 min. The following measures of the copulatory behaviour were recorded by an event recorder (Esterline Angus) for the first ejaculatory series:

1. Latencies. a) Mount and intromission latencies (ML, IL): the time from introduction of the female into the male's cage until the first mount and intromission, respectively. b) Ejaculation latency (EL): the time from the 1st intromission to the 1st ejaculation.

2. Frequencies. a) Mount frequency (MF): the number of mounts in a series. b) Intromission frequency (IF): the number of intromissions in a series.

3. Intervals. a) Mean inter-intromission interval (MIII): the mean interval separating the intromissions of a series. b) Post-ejaculatory interval (PEI): the time from an ejaculation to the next intromission.

4. Total number of ejaculations (mean) during 30 min observation. Results were statistically evaluated using the Student's t-test. Each male rat underwent different mating tests with and without treatments at weekly intervals in a Latin square design. Naloxone was freshly dissolved in bidistilled $H_2O$ and was injected i.p.; control rats received the same volume of bidistilled water.

*Results.* The injection of naloxone, at the dose of 10 mg/kg, did not influence the percentage of rats reaching ejaculation or the mean number of ejaculations achieved during the observation period. However, naloxone significantly altered other measures of the copulatory behaviour. Thus after naloxone treatment, rats ejaculated sooner (shorter ejaculation latency) after fewer intromissions (lower intromission frequency) and with lower rates of intromission (longer inter intromission interval). On the other hand, naloxone did not alter other measures, such as the mount and intromission latencies, post ejaculation interval and mount frequency.

*Discussion.* The results of the present experiments show that naloxone reduces the number of intromissions occurring before ejaculation. It is likely therefore that naloxone lowers the threshold for activation of ejaculatory mechanisms.

Conversely, the fact that naloxone does not influence other measures of the copulatory behaviour, such as the latencies to the 1st mount and intromission, supports the view that ejaculation, mount and intromission are controlled by different neural mechanisms[8]. Mount and intromission latencies are considered measures of sexual arousal and drive[8]. It is likely therefore that since rats used in the present study had a maximal degree of sexual arousal prior to treatment, it was impossible to demonstrate an enhancement in these parameters as was expected from studies in rats with low baseline sexual activity[5]. Yet it is possible that in vigorous copulators a further stimulation of sexual arousal may be reflected by a lowering of threshold of ejaculation.

Naloxone modifies copulatory behaviour in a similar manner as does the treatment with low doses of L-DOPA or apomorphine, a specific stimulant of dopamine receptors[9]. This similarity is, at present, unclear. We have provided evidence that enkephalins increase brain dopamine synthesis by an action on opiate receptors at the dopamine nerve terminals[10]. On the basis of such results, we suggested that activation of these receptors results in a decreased release of dopamine which, in turn, increases dopamine synthesis because of removal of the normal inhibitory effect of the monoamine on presynaptic receptors.

In accordance with this hypothesis, sexual inhibition by

Effect of naloxone on copulatory behaviour of male rats

| Measure | Saline | Naloxone (10 mg/kg i.p.) |
|---|---|---|
| ML (sec) | 98.5 ± 10.74 | 89.2 ± 8.65 |
| IL (sec) | 100.3 ± 13.42 | 111.8 ± 15.34 |
| EL (sec) | 715.5 ± 58.53 | 455.6 ± 26.99* |
| MF | 7.4 ± 1.43 | 5.0 ± 1.05 |
| IF | 18.5 ± 2.18 | 10.0 ± 1.39* |
| MIII (sec) | 36.2 ± 3.42 | 54.4 ± 5.79* |
| PEI (sec) | 460.9 ± 27.90 | 455.7 ± 28.63 |
| Total number of ejaculations | 2.1 ± 0.35 | 2.4 ± 0.44 |

Values are means ± SE from 40 rats. * $p < 0.01$ in respect to control value.

endorphins might be interpreted as a decrease in dopaminergic activity; vice versa, the similarity of naloxone effect with that of dopamine agonists might be explained with an enhanced dopaminergic transmission.
The finding that naloxone shortens the ejaculation latency is of great interest, since it suggests that endogenous endorphins may have an inhibitory role in regulating the ejaculation mechanisms.
If the results obtained in rats apply to other species, then opioid agonists and antagonists might become potentially useful therapeutic agents for ejaculation and other sexual disturbances occurring in man.

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## Levamisole inhibits mineral mobilisation, lactate production and lysosomal enzyme release from cultured bones

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*Summary.* Levamisole added to cultured calvarial bones inhibited spontaneous bone resorption, as indicated by reduced release of calcium and inorganic phosphate to the medium. In addition, levamisole reduced lactate production and release of lysosomal enzymes.

In a final effort to improve the periodontal condition of a patient suffering from both juvenile periodontitis and juvenile diabetes which had responded only poorly to conventional treatment, we used levamisole to try to correct the deficient leukocyte and cellular immune functions. The initial effect of the treatment was encouraging as the inflammatory symptoms were reduced, and during the following 1.5 years no progress of the alveolar bone destruction could be detected by radiological examination. Although the effect was most probably the result of a correction of the immune deficiencies, we considered the possibility that levamisole also had a more direct inhibitory effect on bone resorption with regard to its known inhibitory effect upon alkaline phosphatase[1-3]. We report here the results of a study upon the effect of levamisole (L-tetramisole) on bone resorption in vitro.

*Material and methods.* Paired halves of calvarial bones from 6–7-day-old mice (CsA type), were cultured separately at 37 °C on stainless grids in multi-well dishes (Linbro), containing 2.0 ml of a modification of CMRL 1066 medium (albumin added 0.1% w/v) and gassed with 5% $CO_2$ in air as described earlier[4]. In other series, each mouse was injected with 5 μCi $^{45}Ca$ at least 4 days prior to explantation and the calvarial halves were maintained in separate plastic Falcon dishes containing 5.5 ml of the medium, which was changed daily[4]. In each experiment, one of the calvarial halves was used as a control and the other cultured in the presence of levamisole (a gift from Janssen Division, AB Leo, Helsingborg, Sweden). The drug was dissolved directly in the medium. After culture the media were analyzed for their content of calcium ($Ca^{2+}$)[5], inorganic phosphate ($P_i$)[6], β-glucuronidase[7], β-galactosidase[4,7,8], lactate dehydrogenase (LDH)[9], alanine aminotransferase (ALAT)[10], glucose[11] and lactate[12]. In addition the enzyme activities of the calvaria were analyzed as described[4]. All the enzyme assays included controls containing the test substance, and in no case did the substance affect the measurement of enzyme activity. All enzyme activities were calculated as units/half calvarium, where 1 unit refers to the decomposition of 1 μmole of substrate/min. The radioactivity in the media was analyzed in a liquid scintillation-counter[4] and the morphology of the bones was studied with conventional histological techniques.

*Results.* Levamisole, in doses higher than $10^{-4}$ M, was found significantly to reduce the spontaneous mobilisation of $^{45}Ca$ from prelabelled bones cultured for 24 h. The effect was virtually constant between $5\times10^{-4}$ and $5\times10^{-3}$ M. As shown in figure 1, the inhibitory effect of $10^{-3}$ M levamisole remained unchanged when the culture period was extended to 96 h. When the bones were cultured in the presence of the drug for 24 h and the culture continued in the absence of the drug for further 72 h, almost the same daily reduction of $^{45}Ca$ release was obtained as that seen when levamisole was present during the complete culture period. Levamisole did not influence the release of $^{45}Ca$ from heat-killed bones (data not shown).

The effect of $10^{-3}$ M levamisole on the release of LDH and ALAT from calvarial bones cultured for 24 h

| | LDH (U/bone) | | ALAT (U × $10^{-4}$/bone) | |
|---|---|---|---|---|
| | Control (n = 11) | Levamisole | Control (n = 8) | Levamisole |
| Medium 0-24 h | 32 ± 5 | 53 ± 16* | 30 ± 2 | 41 ± 2** |
| Bone organ 24 h | 297 ± 15 | 275 ± 11 | 37 ± 3 | 42 ± 3* |
| Medium + bone organ | 328 ± 17 | 327 ± 13 | 67 ± 4 | 84 ± 3** |

Values are means ± SEM. n = number of paired experiments. * $p<0.05$; ** $p<0.001$.