Effects of Intracerebroventricular Calcitonin in the Conscious Rabbit

During the experimental work for the assessment of pharmacological and toxicological characteristics of salmon calcitonin, we decided also to make a control of the possible interaction of the hormone with the central nervous system (CNS). Behavioural and electroencephalographic (EEG) changes, and also modifications of the threshold to electric stimulation of dental pulp, were chosen as parameters of the control, recalling that pain relief is one of the reported effects of calcitonin in treated patients.

Salmon calcitonin was available only in limited amount so that, in order to achieve better evidence of an influence of calcitonin on CNS, we decided on inject the hormone directly into the cerebral ventricles of rabbits prepared for the combined determinations of the threshold to painful electric stimuli and the EEG record of activity of different brain areas.

Materials and methods. Experiments were performed using unanaesthetized male adult rabbits weighing 2.5 to 3.0 kg with permanent electrodes implanted by standard stereotaxic technique using the coordinates of MONNIER and GANGLOFF[1] for the following brain areas: nucleus medialis dorsalis thalami (MD), formatio hyppocampalis ventralis (HV), formatio reticularis mesencephali (MRF), cortex motoris (MC), nucleus caudatus (NC). Stainless steel screws served as skull electrodes and ground reference.

The animals were also prepared with chronically implanted electrodes for the analgesimetric evaluation according to CHEYMOL et al.[2] based on the licking reaction elicited by electrical stimulation of the pulp of the upper incisors. In each stimulation schedule, the frequency, pulse width and duration of the stimulus remained constant at 5 Hz, 5 msec and 1 sec respectively; only voltage was varied until licking response was elicited (Palmer electronic square wave stimulator C.V.P. model).

Cannulae were chronically implanted into the lateral ventricle for the intraventricular (IVT) microinjection of calcitonin according to FELDBERG and SHERWOOD[3]. Calcitonin (salmon calcitonin Armour AL 0977) was dissolved in saline buffered at pH 4.3 and injected in doses of 8 or 16 U/kg in the constant volume of 50 µl through a small rubber plate which sealed the cannula above. Control animals received, IVT, only the dissolving solution. The cannula placement was verified histologically at the end of the experiment.

EEG activity was recorded on a Battaglia Rangoni EEG apparatus (mod. Neuro 20) before the beginning of the experiment (1 min of recording every 10×4) and after the IVT injections for 120 min (1 min of recording every 10×12). One lead (NC) was analyzed by means of a voltage integrator (Rossi, Milan).

During the experiment, the rabbits sat in a box which gave them enough space for movements. Blood calcium levels were determined according to RAY SARKAR et al.[4] in samples bled before the experiment and at 30, 60 and 90 min after IVT injections.

Results and discussion. The effect of calcitonin injection into the rabbit brain ventricles on the nociceptive electrical stimulation is reported in Figure 1. While dissolving solution is not effective on the threshold voltage, calcitonin in the dose of 8 U/kg enhances the threshold to the painful stimulus. The antinociceptive action has a rapid onset and progressively increases reaching its maximum 90 min after IVT injection. The analgesic effect still persists by the end of the 2nd hour of observation. If the results are considered on a percentage scale (basal threshold = 100) under the IVT calcitonin the voltage rises up to 500% of basal threshold. Such an increase is very marked since the weight of 8 U of salmon calcitonin corresponds approx. to 2 µg of the polypeptide. In the literature, it is reported[5] that 40 µg IVT of morphine are necessary to induce a 800% threshold increase.

In separate rabbits we have observed, using the 16 U/kg dose of calcitonin, an increase of the threshold to the painful stimuli which was of even higher degree and of longer duration than that observed with 8 U/kg. Such

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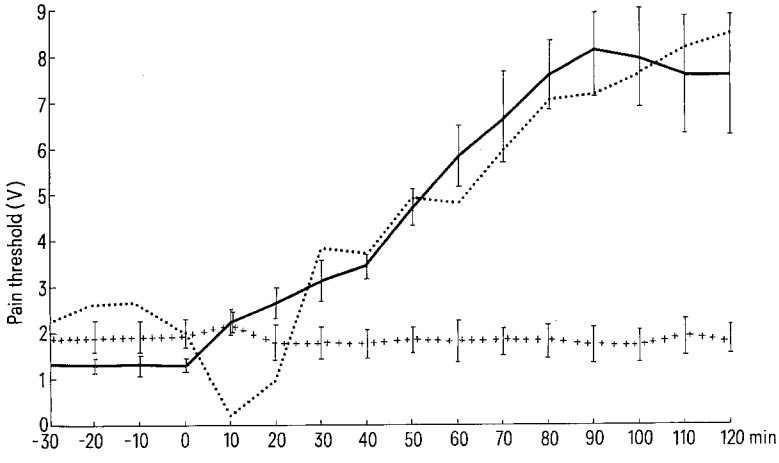

Fig. 1. Threshold for the licking reaction in rabbits given IVT either vehicle (+ + +) or calcitonin (8 U/kg) solution (——) at various times after injection (Mean values and S.D. of 8 animals). The dotted line (. . . .) which represents the integrated EEG voltage recorded from NC is included only to show how changes of electrogenesis parallel analgesia development.

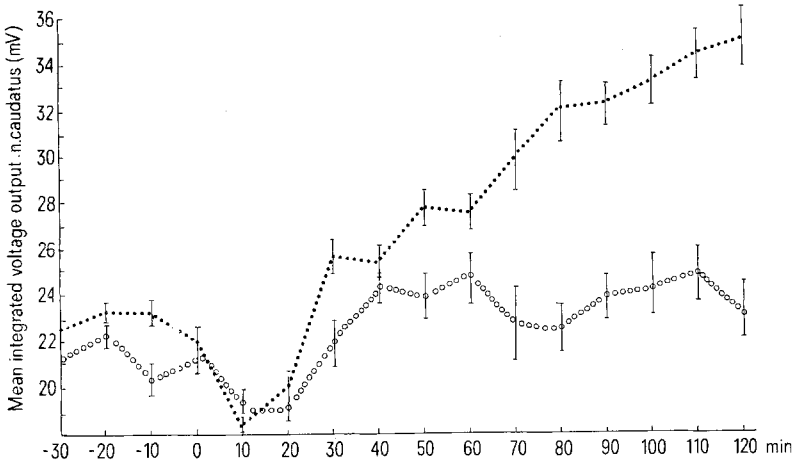

Fig. 2. Effect of IVT vehicle (○○○○) or calcitonin (8 U/kg) injection (....) on conscious rabbit brain electrogenesis at various times after administration (Mean values and S.D. of 8 animals). The integrated EEG recorded from NC is taken as representative of the tracings from all the areas considered.

data would suggest that the effect might be dose-related, but they are not included in Figure 1 because only a few animals have been so far tested.

The calcium levels after IVT calcitonin showed a progressive decrease from the basal mean value of 11.8 ± 1.1 mg/100 ml to 10.6 ± 1.3, 7.7 ± 0.5, 7.5 ± 0.2 mg/100 ml at 30, 60 and 90 min respectively. This fact indicates a leaking of the polypeptide from the brain to the peripheral blood. The significance of changes of calcium ions in analgesia is highlighted also by the reported increase of the analgesic action of morphine and other opiates consequent to a decalcifying agent administration[6]. On the other hand, an involvement of changes of calcium within the neurons induced by calcitonin seems a worthwhile hypothesis, since an influence of the polypeptide on infracellular calcium distribution is suggested as the mechanism through which calcitonin carries out its hormonal action on target cells[7].

As to the results of the EEG studies (Figure 2), we have seen an initial decrease in the average electrogenesis recorded from all the different brain areas which persists about 10 min after the IVT administration both of the vehicle and of calcitonin solution. Then, only in calcitonin-treated rabbits, a phase of progressive increase of EEG voltage follows which reaches its peak approximately in coincidence with the maximal antinociceptive effect (Figures 1 and 2). The integrated voltage values corresponding to the area of the nucleus caudatus plotted in Figure 1 are representative also of other brain areas recorded.

Considering that all the structures from which EEG tracings have been recorded showed similar changes, it is impossible to indicate a specific centre for the action of calcitonin. The parallel increase of all electrical brain potentials and of the thresholds to painful stimuli favours the possibility of a diffuse involvement of neurons' populations by calcitonin, but does not exclude that other areas, not yet investigated, may be more specifically responsive.

Under IVT administration of calcitonin, the conscious rabbits were at first behaviourally excited with signs of hypertonia particularly evident in the neck muscles. Subsequently they showed periodically restlessness with spontaneous running movements.

In conclusion, calcitonin injected into the brain ventricle elicits clear-cut effects including analgesia, the mechanism of which remains largely unknown but deserves further investigation.

Riassunto. La iniezione di calcitonina nei ventricoli cerebrali di conigli non anestetizzati induce analgesia e variazioni elettroencefalografiche con prevalente innalzamento dei potenziali elettrici di diverse aree cerebrali considerate.

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Potentiation of the Effect of Histamine by PGE_2 in the Isolated Perfused Rabbit Kidney and Guinea-Pig Lung

We recently indicated that histamine produces a vasoconstrictor effect on the isolated perfused rabbit kidney and guinea-pig lung acting on histamine H_1-receptors. The vasoconstrictor action of the amine was converted into a vasodilator one by histamine H_1-receptor blockers. The vasodilator action of histamine is due to the stimulation of H_2-receptor since the competitive inhibitors of these receptors (burimamide and metiamide) can significantly inhibit this effect[1,2]. The H_2-receptor blockers added to the perfusion medium cause a potentiation on the vasoconstrictor effect of the amine because of the elimination of masked vasodilator action

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