technique was used for better reproducibility and to shape the pulse. Each monkey was given a unilateral, dorsal-ventral, whole-body irradiation delivered as a short-duration pulse (23 msec width at half-maximum height) of 4000 rads. Student's t test was used to determine the statistical significance of the difference between the means of the histamine values before and after drug treatment or irradiation. Probability values of less than 0.05 were considered significant. Histamine was assayed fluorometrically using the procedure of Shore et al.[8].

Results. The following results were noted. Experiment 1. Within minutes (figure 1) the concentration of histamine in the circulating blood began to increase and became maximal at 3 min. The concentration then began to decrease toward pre-irradiation levels. Experiment 2. Monkeys which are first treated with chlorpheniramine and then irradiation have a histamine concentration which is twice that of untreated animals (figure 1). Experiment 3. The result of partially depleting monkeys of mast-cell histamine can be seen in figure 2. The first dose of 48/80 produced a 298% increase in blood histamine levels. This was accompanied by a marked fall in blood pressure (to 30% of normal). The increase in blood histamine concentration and hypotension became less pronounced with each succeeding day of 48/80 treatment. On the fourth day histamine increased only 17% and the arterial blood pressure decreased only 20%. Experiment 4. Irradiation of monkeys which have been partially depleted of mast-cell histamine produced no significant increase in blood histamine levels (figure 1). Experiment 5. When 48/80 is given to untreated monkeys 20 min after irradiation, there is no significant increase in blood histamine concentration (figure 3).

Discussion. A 4000-rad dose of irradiation releases a significant amount of histamine. When an H_1 blocker, chlorpheniramine, is given before irradiation, the amount of histamine in the circulating blood is greatly increased. The difference in blood histamine concentration following irradiation with and without histamine blockers gives some indication of the amount of histamine that normally goes to receptor sites after release by this dose of radiation. We did not attempt to completely deplete the monkeys of mast-cell histamine with compound 48/80. After 4 consecutive days of treatment (figure 2 and table), the amount of mast-cell histamine released by the 4th dose of 48/80 was greatly reduced. This pseudotachyphylaxis is due to depletion of histamine stores rather than accommodation to 48/80. When the 4 repetitive doses of 48/80 were followed by radiation, there was no measurable increase in blood histamine levels as would be expected if the animals had been depleted of mast-cell histamine. When the animals were first irradiated and then given 48/80 (figure 3) 20 min later, the amount of histamine released was negligible; this is a further indication that 4000 rads of radiation had released most of the mast-cell histamine.

Conclusion. Based on circulating blood histamine levels, the following conclusions can be made: a) a 4000-rad dose of radiation releases a significant amount of histamine; b) the H_1 antagonist chlorpheniramine blocks the attachment to H_1 receptors of most of this histamine; c) the histamine released by 4000 rads of radiation is of mast-cell origin; d) a 4000-rad dose of radiation released most of the mast-cell histamine; and 48/80 given 20 min after irradiation produces only a light increase in circulating histamine levels.

Effects of electrical stimulation of periaqueductal gray matter on evoked potentials recorded in the primary somesthetic cortical areas of the rat

A. Hernández, S. Ruiz, H. Pérez and R. Soto Moyano[1]

Laboratorio de Biofísica y Neurofisiología, Instituto de Nutrición y Tecnología de los Alimentos and Facultad de Medicina Sur, Universidad de Chile, Casilla 15138, Santiago 11, Santiago (Chile), 1 February 1977

Summary. The influence of focal electrical stimulation of PGM on primary cortical evoked potentials elicted by nociceptive peripheral stimulation was studied in anaesthetized and conscious rats. This analgesic electrical stimulation produces an abolition of cortical responses during its application and a significant decrease of the positive and negative amplitude waves after interruption of PGM electrical stimulation. Since these effects were observed in animals under barbiturate anesthesia and in conscious rats, they are interpreted as a supraspinal action on the primary pain pathway.

Deep analgesia, resulting from electrical stimulation of the periaqueductal gray matter (PGM) and other mesencephalic structures, was reported by Reynolds[2] and Mayer et al.[3,4]. Decrease or disappearance of the response to nociceptive stimuli was reported in these publications after stimulation of periaqueductal and periventricular gray matter. Similar results have been reported in the cat[5–7].

It has been postulated that these mechanisms may be similar to the central pharmacological effects of morphine[8], namely, the activation of a descendent inhibitory system which regulates the afferent impulses in the dorsal horn of the spinal cord. On the other hand, it has been shown that morphine has no effect on the amplitude or the latency in the primary pathway[9–12], whereas pentazocine modifies only latency[12].

Up to date the analgesic effect of focal electrical stimulation of the PGM has been studied by algesimetric tech-

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niques. In the experiments reported here, the effects of this analgesic stimulation of the PGM on the primary pain pathway was studied by recording evoked cortical potentials, elicited by peripheral stimulation.

Materials and methods. The experiments were performed in 8 adult male rats weighing 290–320 g, anesthetized with 50 mg/kg of body weight, i.p., of sodium pentobarbital, D-tubocurarine and artificial respiration. 2 conscious rats were also studied; they were initially anaesthetized by ether and subsequently immobilized with D-tubocurarine and maintained under artificial respiration. Experiments were initiated 30 min after the recovery of the rats from the ether action.

Analgesic electrical stimulation was applied by means of a bipolar electrode stereotaxically implanted in the lateral periaqueductal gray matter according to Groot's coordinates, A: 0.0; L: 1.5; V: −2.0 mm. These data were obtained from the Atlas of the rat brain by Pellegrino and Cushman[13]. Electrical shocks were delivered as 200 msec trains of rectangular pulses and separated by 300 msec. Each train consisted of 20 separate, rectangular pulses of 0.2 msec each. The animals were subjected to the following stimulation experiments: a) 5 volts, 10 sec; b) 10 volts, 10 sec; c) 8 volt, 5 min. 30 min, considered as adequate time for recovery, elapsed between experiments. Similar timings and voltages have been used by other investigators[5, 6, 14].

Nociceptive peripheral stimulation consisted of single rectangular electric shocks of 10 V, 0.5 msec and 0.25 Hz, applied to the posterior contralateral limb. The cortical evoked responses were recorded by a monopolar electrode located on the primary somesthetic SI cerebral cortex corresponding to that limb, whose position was assessed by latency time analysis and by the recovery response test to a repetitive stimulation.

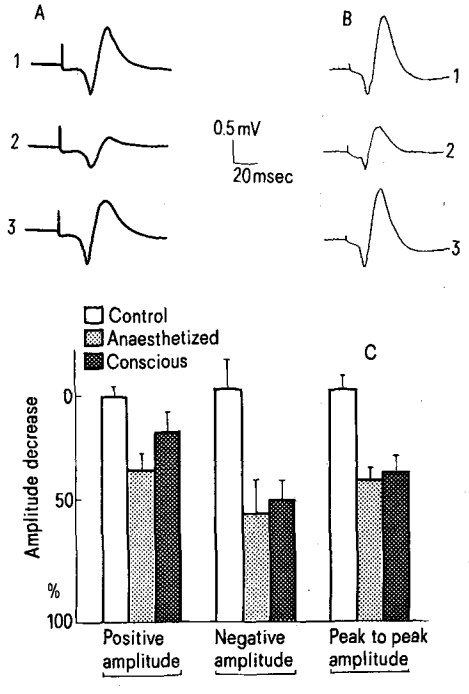
The arithmetic mean of the evoked responses obtained for 15 min previous to the beginning of PGM stimulation was used as the baseline against which all other evoked responses were compared. Results were expressed as variations of the evoked potentials with reference to the control.

Results and discussion. After electrical stimulation of the PGM, a significant decrease of the positive and negative wave amplitudes of the cortical evoked responses was demonstrated in all animals (figure, A and B). For 5 V, 10 sec stimulation, there was a 17.7% decrease of the amplitude of the negative component of the wave ($p < 0.01$). Significant decrease of both positive and negative components were obtained with 10 V, 10 sec stimulation (35.5% for the former, $p < 0.01$, and 40.3% for the latter, $p < 0.01$). The last stimulation experiments, 8 V, 5 min, produced an even greater decrease of the positive amplitude (figure, C).

During application of stimuli, as well as between stimuli, cortical evoked responses were practically abolished. They reappeared when the electrical stimulation of the PGM was interrupted. The experiments carried out on the 2 conscious rats showed comparable results (figure, C).

Electrical stimulation of PGM seems to have an inhibitory influence on the primary pain-sensitive pathway. This phenomenon is characterized by full inhibition of the cortical evoked responses during PGM stimulation. After the periaqueductal stimulation is terminated, partial inhibition of the cortical evoked responses persists for a few minutes. Probably the suppression of the cortical evoked potentials is not inhibition but a phenomenon of occlusion by convergence, similar to that reported by Gauthier et al. on somato-sensitive evoked potentials and Bremer and Stoupel[16] on visual and auditory evoked potentials. The partial inhibition of the cortical evoked responses is probably an inhibitory phenomenon exerted at the level of one of the synaptic connections of the primary pain-sensitive pathway. Possible locations for this action are: a) the dorsal horn of the spinal cord; b) the ventral posterolateral (VPL) nucleus of the thalamus; and c) at a cortical level.

It is known that afferent impulses may be regulated at the intercalar neurons of the spinal cord gelatinous substance of Roland. Evidence of descending inhibitory pathways of supraspinal origin has been provided by Carpenter et al.[17], Taub[18] and Handwerker et al.[19] among others. However, these investigators agree that barbiturate anesthesia depresses this inhibitory system at smaller doses than those used in our experiments. Therefore, the inhibition we observed cannot be explained solely on this basis, since it was also observed in conscious rats.



Amplitude decrease of primary evoked potentials elicited by electrical stimulation on periaqueductal gray matter. *A* Evoked responses of anaesthetized rats. *B* Evoked responses of conscious rats. In both sequences: *1* before PGM stimulation (control); *2* 1 min after supression of 8 V, 5 min of PGM stimulation; and *3* 10 min after supression of PGM stimulation. *C* Percent of decrease of evoked responses recorded during the first min after supression 8 V, 5 min of PGM stimulation. Columns indicate arithmetic means and vertical bars indicate standard errors. $p < 0.01$ in relation to control (Student's t-test).

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At the level of the thalamus, it has been shown that SI cerebral cortex inhibits the progress of primary impulses beyond the VPL nucleus[20,21]. It is possible that PGM stimulation inhibits this thalamic nucleus through cortical activation; such cortical activation may be produced through the ascending activating system of the brain stem. Finally, a directly inhibitory action on primary cortex is also possible through the diffuse projecting system or the specific projection of VPL through a lemniscal-extralemniscal convergence[22,23]. Of the 3 possibilities analyzed here, the first can be excluded, since this inhibitory effect was observed in both anaesthetized and conscious animals.

It is important to emphasize that the inhibition of the negative component on the cortical evoked potential was greater than that of the positive component. This observation does not support the idea of subcortical inhibition, since under these circumstances both components should have been similarly affected. It is also important that inhibition is more evident when longer periods of stimulation are applied. This finding agrees with observations by Melzack and Melinkoff[24] that electrical stimulation of central gray matter has to be applied for about 5 min for maximum analgesia. In addition, the inhibitory action we observed persisted for 2 or 3 min after stimulation was interrupted, with full recovery in 5 min. This persistence of the inhibitory action upon cortical evoked potentials is comparable to the observations on electrical analgesia in rats, performed by other investigators[2-4].

The inhibitory effects reported here may participate in the analgesic mechanism elicited by stimulation of PGM. Unfortunately we do not have confirmatory data that support such a hypothesis.

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Rhythmic variations in the isocitrate dehydrogenase activity in the scorpion, Heterometrus fulvipes (C. Koch)

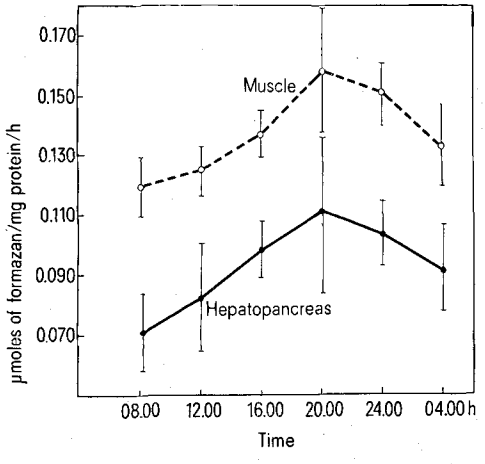
S. Mastanaiah, D. Chengal Raju and K. S. Swami

Departments of Zoology, S. G. S. Arts College and S. V. University, Tirupati-517501, A. P. (India), 4 October 1976

Summary. The activity of isocitrate dehydrogenase was assayed in the pedipalpal muscle and hepatopancreas of scorpion, Heterometrus fulvipes. The enzyme activity showed a circadian rhythmicity with a peak value at 20.00 h in both the tissues.

Rhythmicity in arachnid metabolism has received only limited attention. Diurnal rhythms in various activities like locomotion, poison secretion[1], neurosecretion[2], rate of heart beat, choline esterase activity in heart muscle[3], phosphorylase activity in the hepatopancreas and muscle[4], spontaneous electrical activity in the ventral nerve cord and segmental nerves[5], in the levels of metabolites like blood glucose and hepatopancreatic glycogen[6], have been reported in the scorpion, Heterometrus fulvipes. The above investigations suggest that the biological constituents (metabolites and enzymes) vary in a rhythmic manner during 24 h. This prompted us to study the isocitrate dehydrogenase activity in the pedipalpal muscle and hepatopancreas of the scorpion, Heterometrus fulvipes, as a function of the time of day. The pattern of activity of this enzyme which plays a vital role in citric acid cycle should reveal the pattern of utilization of energy sources for various activities during the course of 24 h period.

Material and methods. The details of collection and maintenance of scorpions, and sampling of tissues were described earlier[6,7]. 10% (w/v) homogenates of tissues were prepared in 0.25 M ice-cold sucrose and centrifuged at 2,500 rpm for 15 min; 0.5 ml of each supernatant (containing 50 mg of tissue) was assayed for the isocitrate dehydrogenase (EC.1.1.1.41) activity level by the method of Kornberg and Pricer[8] with the following modifications.



Isocitrate dehydrogenase activity in the scorpion, Heterometrus fulvipes, as a function of the time of day. Each point represents the mean of 6 estimations. The vertical bars above and below the points indicate the SD limits. The animals were maintained in the laboratory under normal (12 h light/12 h darkness) conditions. The day and night temperatures during the experiment were 36 ± 1 °C and 28 ± 1 °C respectively.

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