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SHORT COMMUNICATIONS

A hypnotic and possible analgesic effect of imidazoleacetic acid in mice*

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IN THE course of screening experiments it was found that 10 male mice fell asleep shortly after imidazoleacetic acid (IMA) was injected i.p. in 0·1 ml of a neutral solution at a level of 4 m-moles/kg. Essentially the same results were obtained in a subsequent experiment with 5 male mice (fasted; 25 g) which had been given the same dose of IMA. In less than 5 min the mice became comatose and lay on one side. When examined at 1 hr after the injection, the mice were colder than normal to the touch. Momentary arousal could be achieved by pinching the tail or by other vigorous handling. One of the mice in this group died during the night, but the other four were alert and eating the following morning and behaved normally for several days of observation.

We have searched the literature and found only one previous report that deals with a biological activity of IMA in which it was found that this substance had an inhibitory action on the stretch receptor neuron of the crayfish, *Pacifastacus leniusculus* (Dana).[1] There appeared to be great specificity in the action of IMA on the stretch receptor neuron, since it was found that the following substances gave negative results: imidazolepropionic acid; 1-methylimidazole-5-acetic acid; histidine; 4-hydroxymethylimidazole; histamine; N-acetyl-L-histidine; histidinol; L-ergothionine; and L-2-thiolhistidine.

Results obtained in further studies of the effects of IMA in mice are described in the remainder of this communication. Preliminary experiments have indicated that IMA also has similar effects in rats and guinea pigs, but not in rabbits.

RESULTS AND DISCUSSION

General findings

Ten groups of 10 fasted mice each were given i.p. injections of IMA in 0·1 ml at the following dose levels: 0·0156, 0·0312, 0·0625, 0·125, 0·25, 0·50, 1, 2, 3, and 4 m-moles/kg respectively. At doses through 0·25 m-mole/kg there were no visible effects in the injected animals. The mice receiving 0·5 m-mole/kg remained upright but showed less spontaneous activity and became somewhat less than normally responsive to tactile and auditory stimulation. At the 1 m-mole/kg level all the animals fell asleep in approximately 30 min; 8 of the 10 regained an upright position within 90 min, while 2 mice awoke only after 6 hr. The mice receiving 3 and 4 m-moles/kg slept 3 to 6 hr. One of the animals receiving the 4 m-moles/kg dose of IMA died 2 hr after the injection. At all times the sleeping mice could be aroused by sufficiently strong pinching at the base of the tail, but immediately after the cessation of stimulation lapsed again into a somnolent state.

Samples from five different synthetic batches of IMA, meeting the usual chromatographic and analytical requirements for purity, were tested. Groups of 5 mice, each maintained in different cages, were given a single i.p. injection at a dose level of 3 m-moles/kg. All the lots of IMA tested were effective. The minimal sleeping time was over 2 hr, while 7 of the animals slept at least 7 hr, the longest period of observation. The following morning all 25 mice appeared to be normal in every respect. Autopsies performed on one animal picked at random from each cage showed the brain and all internal organs to be normal.

Quantitative estimates of analgesic and hypnotic effects in mice

We attempted to assess the analgesic effectiveness of IMA in mice by a modification of the commonly used "hot plate" method.[2] A thermostatically controlled hot plate was set at 55°. A bottomless

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restraining cylinder, 5 inches in height, was made from a tin can. Mice were placed on the hot plate in the cylinder and were observed. The reaction time was measured with a stopwatch from the moment the feet of the mouse touched the hot plate. The endpoint employed in this test[2] is either the licking of the front feet or the climbing or jumping out of the cylinder, whichever occurs first. All other behavioral signs are disregarded. If the response time required in our experiments was less than 30 sec the result was called negative; if it was 30 sec or greater it was considered positive. The animals were not allowed to remain on the hot plate longer than 35 sec. Ten cages of mice were used, each containing eight to ten fasted male mice averaging 25 g in body weight. The reaction times of the mice in each cage were measured, and then the animals were injected with 0·1 ml of neutral solution containing IMA. The control reaction times averaged 10 sec, the range of values being between 1 and 15 sec. The reaction times of all the mice were then remeasured at 30 and 120 min after the injection. In those instances in which positive results were obtained, the results at the period giving the maximal number of positive responses were employed for the points shown in Fig. 1.

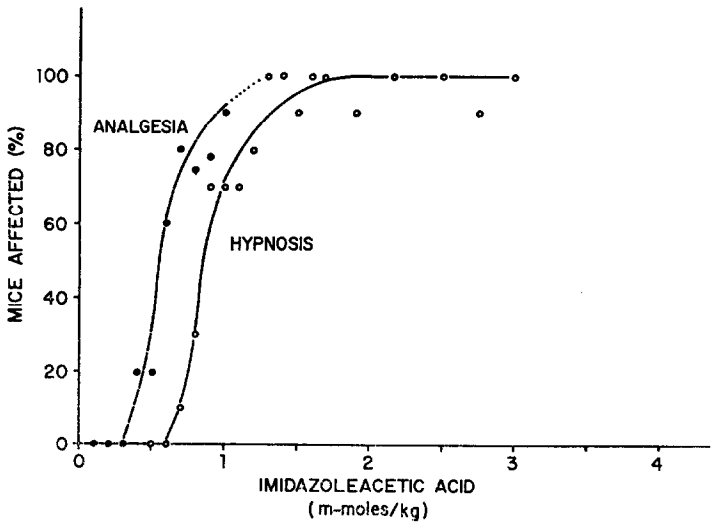

FIG. 1. Analgesic and hypnotic effects of different doses of imidazoleacetic acid.

When IMA was tested at hypnotic levels, we noted that there always was a period during which there was impairment in the righting reflex prior to sleep. It was possible to detect this effect either by direct observation of the animals or by turning them over and noting whether or not they returned to a normal position. It was decided to employ the loss of the righting reflex as an indicator of the onset of the hypnotic effect of IMA. Eighteen groups of ten mice each were tested at dose levels of IMA ranging from 0·5 to 3 m-moles/kg. The percentages of the animals in which a hypnotic effect was produced at the various doses are shown in Fig. 1. It is interesting that in this series of experiments no analgesic effects were noted at 0·1–0·3 m-mole/kg and that a dose of IMA (0·6 m-mole/kg) which was 60 per cent effective in the analgesic test was below the level producing a hypnotic effect. Since it was noted previously that the spontaneous activity of mice was decreased at 0·5 m-mole/kg, it would be difficult by these tests to distinguish a true analgesic effect from a general depression at levels of 0·5 m-mole and above. The results in Fig. 2 show that a relationship exists between the average time after injection of IMA required for loss of the righting reflex and the dose of IMA employed. No such relationship was found between the dose of IMA and the total sleeping time. All the mice that fell asleep in this series of experiments slept for at least 2 hr.

Specificity of effect of imidazoleacetic acid

It was of interest to determine whether other compounds with the imidazole ring would have effects in mice comparable to those observed with IMA. Negative results were obtained with imidazolepropionic acid (dihydrourocanic acid) in experiments with four groups of five mice each injected

with 1, 2, 3, or 4 m-moles/kg. Experiments in 40 mice with histidine at 4 m-moles/kg also were negative. The following substances tested at 3 m-moles/kg in the indicated number of mice also produced none of the effects noted with IMA at this level: imidazole, 10; 4-hydroxymethylimidazole, 10; 1-methylimidazole-4-acetic acid, 7; imidazolepyruvic acid, 10; imidazoleacrylic acid, 10; 1-acetylimidazole, 10; 4-imidazole carboxylic acid, 10; and 4,5-imidazole dicarboxylic acid, 10. Therefore,

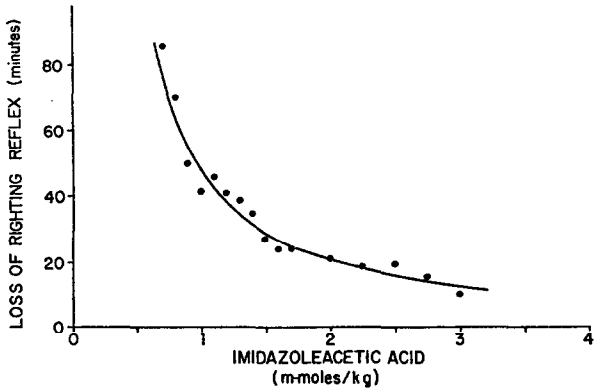

FIG. 2. Average times required for loss of righting reflex as a function of dose of imidazoleacetic acid.

it appears that considerable structural specificity must be associated with the biological effects of imidazoleacetic acid in mice.

Models show that in IMA the carboxyl group could form a hydrogen bond with the ring nitrogen in the 3-position. The molecule then could appear like a fused ring system, one of the surfaces of which is almost planar. Studies are under way of various physical parameters which might be related to the physiological effects of IMA and of the distribution, metabolism, and biochemical effects of the substance.

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Blocking effect of puromycin, ethanol, and chloroform on the development of tolerance to an opiate*

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PUROMYCIN has been found to block retention of a conditioned avoidance response,[1] suggesting that this learning process is associated with protein formation. Recently Cohen *et al.*[2] found that tolerance

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