Pharmacological and Toxic Effects of Kryptopyrrole in Mice

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In view of the established correlations between clinical psychiatric measures and kryptopyrrole (2, 4-dimethyl-3-ethylpyrrole), and the paucity of knowledge concerning the mechanism underlying these correlations, a preliminary pharmacological investigation of the substance was undertaken, utilizing mice as the experimental animal.

In mice, kryptopyrrole was found to have pronounced general behavioral, hypnotic, and hypothermic effects.

Introduction

Kryptopyrrole (2,4-dimethyl-3-ethylpyrrole) has been found in the urine of some patients with psychotic symptoms as reported by Irvine et al. (1969) and later verified by Sohler et al. (1970).

Preliminary findings indicate that patients with acute intermittent porphyria excreted increased amounts of urinary kryptopyrrole during acute attacks associated with neuropsychiatric symptoms (Irvine and Wetterberg, 1971). These symptoms of acute porphyria are of special interest since they are sometimes of schizophrenic type (Waldenstrom, 1937; Peters, 1962) or constitute a porphyria mental syndrome with moderate depression, transitional confusion, visual hallucination, and neurological signs (Wetterberg, 1967).

The finding of kryptopyrrole in the urine of psychotic and porphyric patients prompted a study of the pharmacological effects of this substance. In the present study, general behavioral, hypnotic, and hypothermic effects of kryptopyrrole in mice are reported.

This paper was presented at the joint meeting of The American Schizophrenia Association, The Canadian Schizophrenia Association and The Schizophrenia Association of Great Britain; London, England, September 28-30,1971

MATERIALS AND METHODS

Animals

One hundred and forty mice (Naval Medical Research Institute strain, obtained from Anticimex, Stockholm) were used. The animals' body weight ranged between 20.0-22.3 g. They were fasted over night and received water **ad libitum.**

Dose and Administration

Kryptopyrrole, purchased from Aldrich Chemical Company, was dissolved in propylene glycol and given intraperitoneally (i.p.) in a volume of 0.1 ml at the dose levels: 0.0078, 0.0312, 0.0625, 0.125, 0.25, 0.5, 1, 2 and 4 millimoles (m-moles)/kg respectively. Ten mice were injected at each dose level. The mice were injected to the right of the umbilicus.

Behavioral Observations

The reactions of the mice after i.p. injection were checked by direct observation of the animals for six hours, and the latency to ataxia was recorded. The loss of righting reflex was detected by turning the mice on their backs and noting whether they returned to a normal position or not.

Hot Plate Method

The analgesic effect was assessed by a modification of the hot plate method of Jans-sen and Jageneau (1957). A thermostatically-controlled hot plate was set at 55°C. A bottomless restraining cylinder 10 cm in height

was placed on the hot plate, and the mice were placed on the hot plate (in the cylinder) and observed. The reaction time was measured by a stop watch from the moment the feet of the mouse touched the hot plate. The end point employed in this test is either the licking of the front feet or the climbing or jumping out of the cylinder, whichever occurs first. If the response time on the hot plate to licking, climbing, or jumping was less than 20 seconds, it was considered that no analgesic effect was presented and the results were called negative; if the response time was 20 seconds or longer, the test result was considered positive. The animals were not allowed to remain on the hot plate more than 25 seconds. The reaction time of all mice in each cage was measured before i.p. injection of kryptopyrrole, and were remeasured at 30 and 120 minutes after the injection.

Measurement of Body Temperature

The body temperature was measured with an electric thermometer (accuracy: O.10C Ellab, Copenhagen) before, and 180 minutes after, injection of kryptopyrrole. In a second experiment, the body temperature of 10 control animals and eight mice receiving 0.5 and 1 m-moles/kg respectively, and five mice receiving 2 m-moles/kg of kryptopyrrole, was recorded every hour for six hours. The thermocouple was placed in the colon 20 mm from the anal orifice. The room temperature during the experiment was 22^{O}C.

RESULTS Behavioral Observations

At doses below 0.25 m-moles/kg of kryptopyrrole, no gross effects were observed. The mice receiving 0.25 m-moles/kg remained upright but showed less spontaneous activity and became somewhat less than normally responsive to tactile and auditory stimulation. Three of the 10 animals showed ataxia and intermittent propulsive movements within 30 minutes after injections. At the 0.5 m-moles/kg dose level, all the animals showed ataxia within 30 minutes and behavioral sleep within 120 minutes.

The animals receiving 2 and 4 m-moles/kg did not respond 120 minutes after injections even to vigorous stimuli, e.g. arousal by tail pinching. No tendency to convulsions was recorded at any dose levels.

Fifty percent of the animals receiving 1.0 m-mole and all the animals receiving 2 and 4 m-moles/kg were dead in the morning the day after the experiment.

Reaction Time on Hot Plate

The control reaction time averaged 3 seconds, the range being 1-9 seconds. The positive results recorded at the time period (30 or 120 minutes after injection) giving maximal number of positive responses are shown in Figure 1. It was noted at the 0.5 m-moles/kg level that all the animals showed ataxia but were still able to climb out of the cylinder. At 1 m-mole/kg three animals did not lick their front feet and did not climb out of the cylinder, two animals jumped out and the remaining five only licked their front feet. At 2 and 4 m-moles/kg, all of the animals were ataxic and did not lick their feet or climb out of the cylinder.

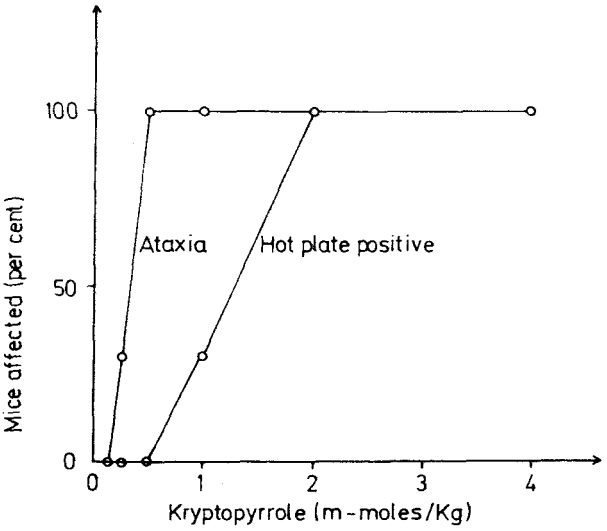

FIGURE 1

Ataxia and positive reaction of hot plate test on different doses of kryptopyrrole.

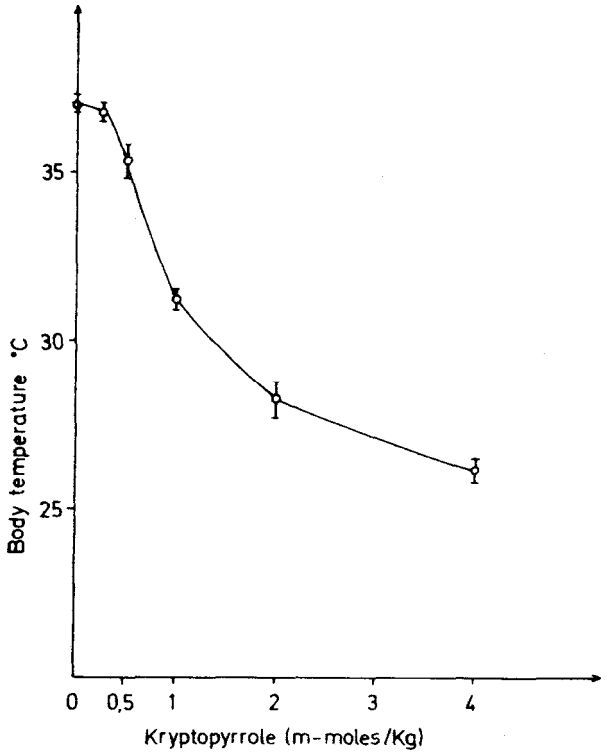

FIGURE 2

Body temperature three hours following injections of different doses of kryptopyrrole. Each point and bar on the graph represents the mean and standard error for 10 mice.

Hypothermic Effect

Kryptopyrrole injections decreased the body temperature, and the results in Figure 2 show a dose response relationship between kryptopyrrole and the decrease in body temperature. The time courses of the lowering of body temperature of three different dose levels, 0.5, 1, and 2 m-moles/kg, show that the hypothermic effect developed rapidly, and following 2 m-moles/kg the temperature decreased 6 degrees within the first 60 minutes after injection (Figure 3).

Discussion

In the present work, the general behavioral, hypnotic, and hypothermic effects of kryptopyrrole were tested. The progressive increase in sedation, with increasing doses, is in agreement with earlier findings of the effect of kryptopyrrole in rabbits (Sohler et al., 1970) and rats (Irvine and Zdanivsky, 1971).

The true nature of behavioral sleep of the mice receiving 0.5 m-mole/kg or more of kryptopyrrole has to be further evaluated and studied in relation to electroencephafographic findings, particularly signs of sleep.

The present data indicate that kryptopyrrole may also have an analgesic effect. Neuromuscular apparatus impairment

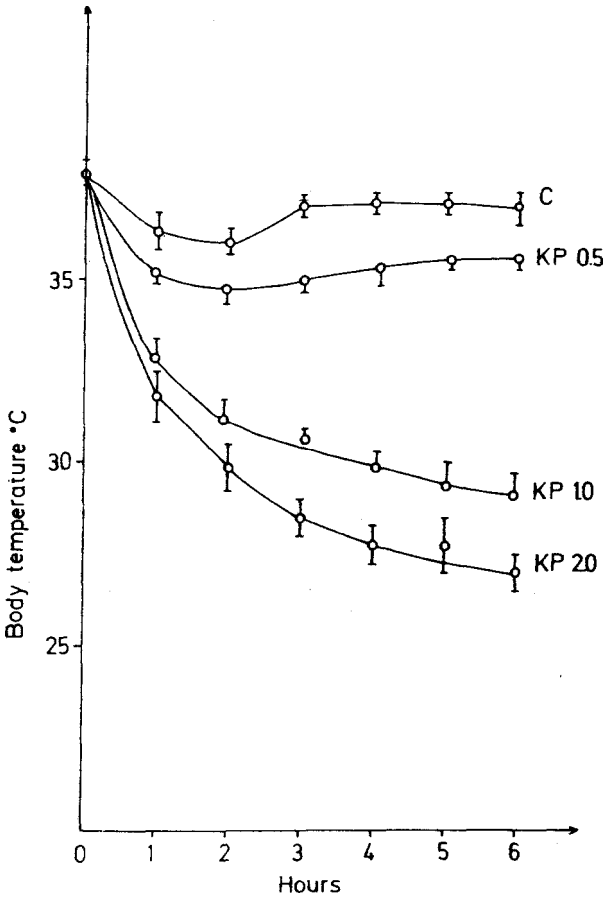

FIGURE 3

Body temperature every hour for six hours, of control mice given propylene glycol (C) and mice given 0.5, 1.0, and 2 m-molesAg of kryptopyrrole (KP) at 0 time.

Each point and bar on the graph represents the mean and standard error for five to eight mice.

developed at a lower dose level than the analgesic effect, since five rats were unable to climb the cylinder in the hot plate test even though they reacted to the heat stimulus by licking their front feet.

The mechanism causing the rapid hypothermic effect of kryptopyrrole, with 6° lowering of body temperature within the first hour, is not evident.

The present study shows that kryptopyrrole causes neurological symptoms in mice, and raises the question as to whether the schizophrenic syndrome and the severe neuropsychiatric symptoms found during attacks of acute intermittent porphyria in humans are in some way related to kryptopyrrole formation.

This makes it important to further study other properties and effects of kryptopyrrole.

ACKNOWLEDGEMENTS

The author is grateful to Donald G. Irvine who first called my attention to kryptopyrrole.

I wish to thank Sune Carlsson and Lars Hoflund for technical assistance.

This study was supported by grant number K71-21 X-3371-01A from the Swedish Medical Research Council.

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