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Role of 5-hydroxytryptamine in ketamine-induced hypothermia in the rat

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Summary

1. Intraperitoneal injection of graded doses of ketamine produced a dose-dependent fall in body temperature of rats. Similarly, intracerebral injection of much smaller doses produced hypothermia.

2. Pretreatment of the rats with *p*-chlorophenylalanine (PCPA) greatly attenuated the hypothermic response to ketamine whereas the intraperitoneal injection of 5-hydroxytryptophan in PCPA-treated rats restored the hypothermic effect of ketamine.

3. Depletion of the brain monoamines by reserpine completely prevented the ketamine-induced hypothermia. Treatment with sodium diethyldithiocarbamate (DEDTC), however, did not modify the hypothermic effect of ketamine.

4. Pretreatment of the rats with pargyline potentiated the ketamine-induced hypothermia.

5. Depletion of brain monoamines by reserpine in combination with inhibition of noradrenaline biosynthesis (DEDTC) resulted in a long lasting fall in temperature which was not modified by ketamine.

6. When the ambient temperature was raised from 26° C to 32° C, ketamine-induced hypothermia was much reduced and superimposed on a hyperthermia which occurred in all animals.

7. It is concluded that ketamine produces hypothermia in rats possibly through the release of 5-hydroxytryptamine in the hypothalamus and that this effect is similar in some respects to that produced by morphine in non-tolerant rats.

Introduction

Feldberg & Myers (1964b) have suggested that anaesthetics affect body temperature by releasing the monoamines in the anterior hypothalamus. In cats and dogs in which noradrenaline lowers and 5-hydroxytryptamine (5-HT) raises body temperature, when acting on the anterior hypothalamus (Feldberg & Myers, 1964a; Feldberg, Hellon & Myers, 1966), anaesthetics produce a fall in body temperature. This fall in temperature was explained by release of noradrenaline; release of 5-HT was not excluded but the action of noradrenaline was thought to predominate. In the rat, however, both noradrenaline and 5-HT lower body temperature when

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acting on the hypothalamus (Feldberg, 1968), although noradrenaline may produce a rise (Feldberg & Lotti, 1967b).

In our laboratory, the use of ketamine (2-orthochlorophenyl, 2-methylamine cyclohexanone) as an anaesthetic agent in rats was shown to produce hypothermia. It was thought worthwhile to investigate the nature of this hypothermic response and to find out whether or not it could be modified by drugs which affect the synthesis of brain monamines.

Methods

Female albino rats weighing 150–250 g were used. The experiments were carried out at room temperature ranging between 25 and 26° C. Food and water were withdrawn before the first measurement of body temperature which was measured by electrical thermocouples (Ellab electric universal thermometer type T.E. 3) inserted 4 cm into the rectum. Ketamine was injected intraperitoneally in graded doses ranging between 20 and 100 mg/kg. It was also injected intracerebrally as described by Cox & Osman (1970) in a dose of 0·5 mg/rat contained in 50 μl with a sterile tuberculine syringe. The site of injection was within 5 mm of either side of the midline on a line drawn through the anterior base of the ears. *p*-Chlorophenylalanine (PCPA) was injected subcutaneously as described by Koe & Weissman (1966) as a very fine saline suspension in a dose of 320 mg/kg on the first day followed by 100 mg/kg on both the second and third days, and the rats were injected with ketamine on the fourth day. Sodium diethyldithiocarbamate (DEDTC) was injected intraperitoneally in a single dose of 400 mg/kg 2 h before ketamine injection. Reserpine was injected intraperitoneally in a single dose of 5 mg/kg 24 h before ketamine injection. Pargyline was injected intraperitoneally in a dose of 50 mg/kg daily for 3 successive days and ketamine was injected on the fourth day. Control animals received 0·9% w/v NaCl solution (saline). Other drug treatment will appear in the results.

Drugs

The following drugs were used: Ergotamine tartrate (Sandoz), (±)-5-hydroxytryptophan (Koch-Light), ketamine hydrochloride (Parke Davis), methysergide (Sandoz), *p*-chlorophenylalanine (Sigma), pargyline hydrochloride (Abbott), reserpine (Ciba) and sodium diethyldithiocarbamate (B.D.H.). The doses given in the text refer to the salts.

Results

In rats the intraperitoneal injection of graded doses of ketamine resulted in a dose-dependent decrease in body temperature (Figure 1). Ketamine in a dose of 20 mg/kg and in lower doses did not cause any significant change in body temperature ($P>0·1$). However doses of 50 and 100 mg/kg produced a significant lowering of body temperature ($P<0·005$) and the greatest fall in temperature was caused by 100 mg/kg. This last dose was used as the test dose in subsequent experiments. Hypothermia was maximal 1 h after injection of ketamine and temperature returned to normal in the next 3 hours. Intracerebral injection of much smaller doses of ketamine (0·5 mg/rat) resulted in a significant lowering of body temperature

($P<0{\cdot}005$); however, the time course for the hypothermia was the same as with the intraperitoneal injection (Figure 1).

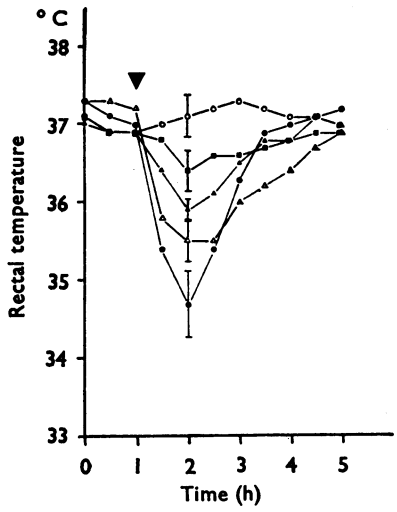

FIG. 1. Rectal temperature of rats. Time-course of response curves for ketamine injected at the arrow. Each value is the mean of six observations except the 100 mg/kg dose where 10 rats were used. The vertical lines indicate S.E.M. ○——○, Saline. Doses of ketamine: ■——■, 20 mg/kg, i.p.; ▲——▲, 50 mg/kg, i.p.; ●——●, 100 mg/kg, i.p.; △——△, 0·5 mg/rat intracerebrally.

Anaesthesia and analgesia were poorly maintained with the 20 mg/kg dose but with the 50 and 100 mg/kg dose both anaesthesia and analgesia were well maintained. Reflex movements, however, were not abolished during anaesthesia.

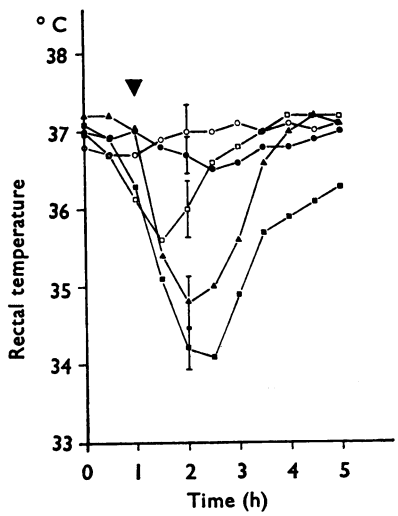

FIG. 2. Rectal temperature of rats. Effect of pretreatment with *p*-chlorophenylalamine (PCPA) on the ketamine-induced hypothermia. At the arrow ketamine (100 mg/kg) was injected i.p. into rats pretreated with PCPA (●——●), PCPA+5-hydroxytryptophan (5-HTP) (■——■) or saline (▲——▲). Saline was injected at the arrow into rats pretreated with PCPA (○——○) or PCPA+5-HTP (□——□). Doses as in text. Each curve represents the mean responses from 6 rats. The vertical lines indicate S.E.M.

Pretreatment with p-*chlorophenylalanine*

Intraperitoneal injection of ketamine (100 mg/kg) in PCPA-treated rats did not result in any significant fall in body temperature when compared with rats pretreated with PCPA and injected with saline ($P>0.1$) (Fig. 2). When rats pretreated with PCPA were injected with 5-hydroxytryptophan (5-HTP, 100 mg/kg intraperitoneally) 30 min before administration of ketamine, the hypothermic response to ketamine was restored (Fig. 2). The injection of 5-HTP in rats pretreated with PCPA resulted in a non-significant fall in body temperature ($P>0.05$) which returned almost to normal within 90 minutes.

Pretreatment with reserpine and/or sodium diethyldithiocarbamate

Pretreatment of rats with reserpine completely prevented the ketamine-induced hypothermia (Fig. 3). Reserpine treatment by itself did not result in any change in body temperature. Pretreatment of rats with DEDTC did not prevent the ketamine-induced hypothermia ($P<0.001$). In another group of rats the combined treatment with reserpine and DEDTC resulted in a long lasting fall of body temperature which was not modified when ketamine was injected 90 min after DEDTC (Figure 3).

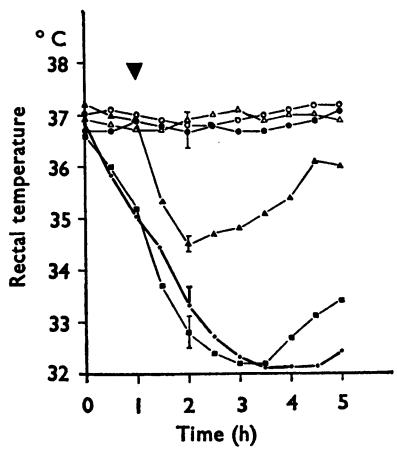

FIG. 3. Rectal temperature of rats. Effect of pretreatment with reserpine and/or sodium diethyldithiocarbamate (DEDTC) on the ketamine-induced hypothermia. At the arrow, ketamine (100 mg/kg) was injected i.p. into rats pretreated with reserpine (●——●), DEDTC (▲——▲) or reserpine+DEDTC (■——■). Saline was injected at the arrow in rats pretreated with reserpine (△——△), DEDTC (○——○) or reserpine+DEDTC (●——●). Doses as in text. Each curve represents the mean responses from 6 rats. The vertical lines indicate S.E.M. The S.E.M. of the DEDTC+saline group=0·22.

Pretreatment with pargyline

Pretreatment of rats with pargyline did not result in any significant change in body temperature. The intraperitoneal injection of ketamine (100 mg/kg) in the pargyline pretreated rats resulted in a significant long lasting fall of body temperature ($P<0.001$) (Fig. 4). The temperature remained at a low level for more than 4 hours.

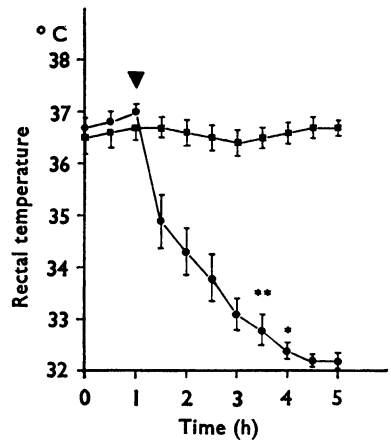

FIG. 4. Rectal temperature of rats. Effect of pretreatment with pargyline on the ketamine-induced hypothermia. Pargyline (50 mg/kg) was injected i.p. daily for 3 successive days. Ketamine (100 mg/kg) was injected at the arrow in one group of rats (●——●), the other group was injected with saline (■——■). Each curve represents the mean responses from 6 rats. The vertical lines indicate S.E.M. At the asterisk rats died in hypothermia.

Pretreatment with methysergide or ergotamine

Intraperitoneal injection of either methysergide (1 mg/kg) or ergotamine (0·5 mg/kg) into rats 30 min before the injection of ketamine failed to modify the hypothermic response.

Effect of raising the ambient temperature

When the environmental temperature was raised from 26° C to 32° C, the ketamine-induced hyothermia was much reduced and superimposed on a hyperthermia which occurred in all animals (Fig. 5). The temperature of the ketamine treated rats was significantly lower than that of the control rats 1 h after ketamine injection ($P<0{\cdot}05$) but was not significantly higher 90 min later ($P>0{\cdot}10$).

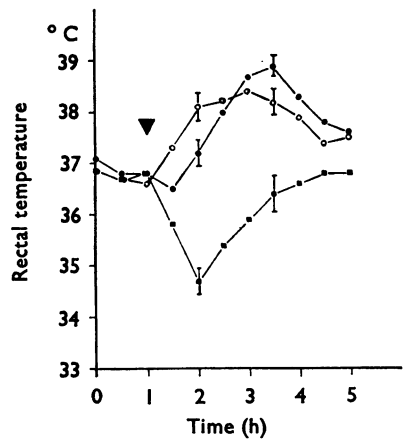

FIG. 5. Rectal temperature of rats. Effect of raising the ambient temperature on the ketamine-induced hypothermia. Ketamine (100 mg/kg) was injected i.p. at the arrow into rats maintained at 26° C (■——■) and 32° C (●——●). ○——○, Saline-treated rats maintained at 32° C. Each curve represents the mean responses from 5 rats. The vertical lines indicate S.E.M.

Discussion

The experimental results reported in this study show that intraperitoneal injection of graded doses of ketamine result in a dose-dependent fall in body temperature in rats. This is in agreement with the results reported by Feldberg & Myers (1964b) for anaesthetics. Also intracerebral injection of much smaller doses of ketamine (which are ineffective after systemic administration) produced a significant fall in body temperature of the rats which suggests that ketamine acts mainly centrally.

Feldberg & Myers (1964b) have suggested that the fall in temperature which occurs during anaesthesia is due to the release of monoamines in the anterior hypothalamus. The results obtained in the present investigation suggest that the release of brain 5-HT might be involved in the ketamine-induced hypothermia. This is supported by the fact that pretreatment with PCPA, a drug which selectively depletes brain 5-HT (Koe & Weissman, 1966), greatly attenuated ketamine hypothermia. In addition, injection of 5-HTP into PCPA-treated rats restored the hypothermic effect of ketamine. Similarly, reserpine pretreatment which depletes both brain 5-HT and noradrenaline (Pletscher, Shore & Brodie, 1956; Holzbauer & Vogt, 1956) completely prevented ketamine hypothermia. However, treatment with DEDTC, a drug which prevents the formation of noradrenaline from dopamine by inhibiting the enzyme dopamine β-hydroxylase (Collins, 1965; Carlsson, Lindqvist, Fuxe & Hökfelt, 1966. Aigner, Hornykiewicz, Lisch & Springer, 1967. Maj & Vetulani, 1970), did not modify ketamine hypothermia. Moreover, reserpine pretreatment in combination with inhibition of noradrenaline biosynthesis (DEDTC) resulted in a long lasting fall in temperature which was not modified by ketamine injection. This excludes any role of noradrenaline as a possible mediator in ketamine hypothermia.

Hypothermia in the rat was produced following the injection of 5-HT either intraventricularly (Feldberg & Lotti, 1967b) or intracisternally (Bruinvels, 1970). Ketamine-induced hyothermia appears to be mediated centrally and not peripherally since it was not antagonized by either methysergide or ergotamine.

Ketamine injection in pargyline pretreated rats resulted in a highly significant fall in body temperature which lasted for a long time. Similar results were obtained with iproniazid (Osman, unpublished observation). This potentiation by pargyline could be explained by the fact that in the rat both 5-HT and noradrenaline lower body temperature when acting on the hypothalamus (Feldberg, 1968). If it is assumed that only 5-HT but not noradrenaline is released by ketamine, then the undestroyed 5-HT as a result of MAO inhibition will produce this deep fall in temperature. The possibility of an additive effect of the already elevated noradrenaline from MAO inhibition could not be excluded. It is worth mentioning here that MAO inhibitors have been shown to reverse the hypothermic response of anaesthetics in cats and dogs (Feldberg & Lotti, 1967a. Summers, 1969). This difference in results could be explained on the basis of species variation. In cats and dogs, 5-HT raises and noradrenaline lowers body temperature (Feldberg & Myers, 1963, 1964a; Feldberg, Hellon & Myers, 1966).

When the environmental temperature was raised to 32° C, ketamine hypothermia was much reduced and superimposed on a hyperthermia which occurred in all animals. This is in agreement with the results reported by Shemano & Nickerson (1958) who injected 5-HT subcutaneously in rats.

Ketamine was reported to produce conspicuous analgesia, amnesia and what is called ' dissociative anaesthesia ' and is believed to act mainly on the cerebral cortex (Virtue, Alains, Mori, Lafargue, Vogel & Metcalf, 1967). There are some points in common in this respect between ketamine and morphine, both being analgesics and recently morphine was found to produce hypothermia in non-tolerant rats which was mediated through the release of 5-HT (Oka, Nozaki & Hosoya, 1972).

It is concluded that ketamine-induced hypothermia in rats may be mediated through the release of 5-HT in the anterior hypothalamus, and that this hypothermia is similar in some respects to that produced by morphine in non-tolerant rats.

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