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Role of noradrenaline and 5-hydroxytryptamine in tetrahydronaphthylamine-induced temperature changes in the rat

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Summary

1. Intraperitoneal administration of graded doses of tetrahydronaphthylamine (THN) to rats caused a dose dependent decrease in body temperature.

2. Intracisternal injection of graded doses of THN induced hypothermia, and implantation of crystalline THN rostral to the medial preoptic area and caudal to the striatum, caused hyperthermia.

3. Pretreatment of the rats with a MAO inhibitor changed the hypothermia into hyperthermia.

4. Intraperitoneal injection of 5-hydroxytryptophan caused a hypothermia which could be reversed into hyperthermia when the rats were pretreated with a MAO inhibitor.

5. Pretreatment with parachlorophenylalanine enhanced the THN-induced hypothermia.

6. Depletion of brain monoamines by Ro-4-1284 in combination with an inhibition of the biosynthesis of noradrenaline (diethyldithiocarbamate) changed the THN-induced hypothermia into hyperthermia.

7. It is concluded that THN affects body temperature in rats by two central mechanisms, viz. a decrease mediated by noradrenaline, probably in the hypothalamus, and an increase which might be mediated by 5-hydroxytryptamine rostral to the medial preoptic area.

Introduction

The actions of 1,2,3,4-tetrahydro-2-naphthylamine (THN) became of interest at the beginning of the century. As early as 1889, Stern had observed its hyperthermic effect in rabbits and dogs after subcutaneous injection. Both hypo- and hyperthermic responses, depending on the environmental temperature, have been described in rats (Giaja & Dimitrijevic, 1933).

Following the suggestion by Feldberg & Myers (1963) that body temperature is regulated by the balance of the actions of the monoamines noradrenaline (NA) and 5-hydroxytryptamine (5-HT) released in the hypothalamus, experiments were carried out to find out if the changes in temperature induced by THN in the rat

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were mediated by these monoamines and were thus dependent on their availability. Different procedures were used to investigate this problem. The temperature responses to THN given by different routes—injected intraperitoneally, intracisternally, or implanted as crystals near the preoptic area—were compared. In addition the effect of inhibition of the MAO by nialamide was examined on the responses, not only to intraperitoneal THN, but also to intraperitoneal 5-hydroxytryptophan (5-HTP). Finally, the effect of inhibition of tryptophan hydroxylase as well as the combined effect of depletion of the monoamines with inhibition of NA biosynthesis were studied on the temperature response to intraperitoneal THN. Inhibition of tryptophan hydroxylase was produced by parachlorophenylalanine (PCPA) depletion of the monoamines by RO-4-1284 and inhibition of NA synthesis by diethyldithiocarbamate (DEDTC) which acts by inhibiting the dopamine-β-hydroxylase (Carlsson, Lindqvist, Fuxe & Hökfelt, 1966). Some of the results were presented at the Dutch Federative Meeting of Medical-Biological Societies, Utrecht (Kemper & Bruinvels, 1969).

Methods

Male albino rats, weighing 100–110 g were placed in individual cages 16 h before treatment, in a room at constant temperature (24·5 ± 0·5° C). Food and

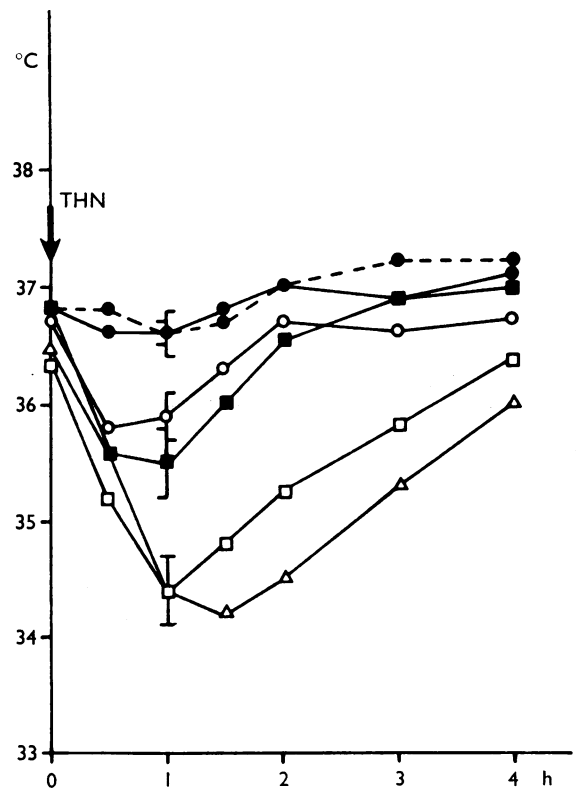

FIG. 1. Rectal temperature of unanaesthetized rats. Dose-response curves for intraperitoneal THN. Each value is the mean of six observations and the standard errors are indicated. (●--●), Saline. Doses of THN: (●——●), 2·5 mg/kg; (○——○), 5·0 mg/kg; (■——■), 10·0 mg/kg; (□——□), 20·0 mg/kg; (△——△), 40·0 mg/kg.

water were withdrawn before the first measurement of body temperature, which was measured with a Telethermometer (Yellow Spring Co.) by inserting its probe 3 cm into the rectum. Drugs given intraperitoneally were injected in a volume of 1 ml. PCPA (3·16 mg/kg) was administered as an acid solution in a volume of 2 ml by stomach tube as described by Tenen (1967). Controls received the vehicle only. The injection of THN into the cisterna magna was made under light ether anaesthesia. Crystalline THN was tamped into a stainless steel cannula, with an outer and inner diameter of 0·6 and 0·3 mm respectively. The cannula was introduced stereotaxically into the brain under light ether anaesthesia (Ernst & Smelik, 1966). The position of the cannula was A 8·5, L 3·8 and D −2·0 mm with an angle of 5°. When the tip of the cannula had reached the desired position, the THN was delivered by pushing a stilette down the cannula. Control rats were treated in the same manner with an empty cannula. The difference of significance from controls was determined by Student's *t* test.

The following substances were used: 1,2,3,4-tetrahydro-2-naphthylamine HCl (Suchardt), (±)-5-hydroxytryptophan (Fluka), nialamide (Pfizer), parachlorophenylalanine (Pfizer), Na-diethyldithiocarbamate (Noury-Baker) and 2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo-(a)chinolizin (Ro-4-1284) (Hoffman La Roche). The doses given in the text refer to free bases or acids.

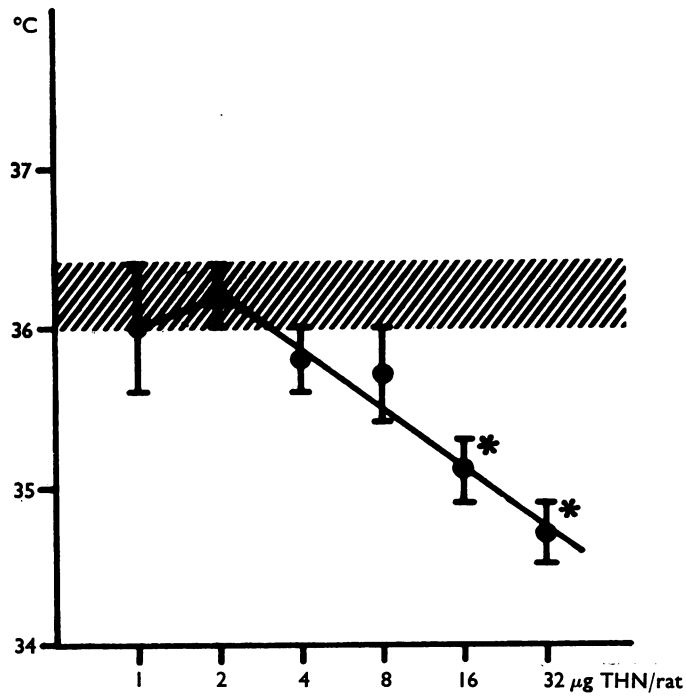

FIG. 2. Rectal temperature of unanaesthetized rats 10 min after injections of 1–32 μg THN into the cisterna magna. Each value is the mean of seven experiments and the standard errors are indicated. The points marked with an asterisk are significantly different from the controls ($P<0·01$). The shaded area gives the temperature 10 min after intracisternal injections of 0·9% NaCl solution.

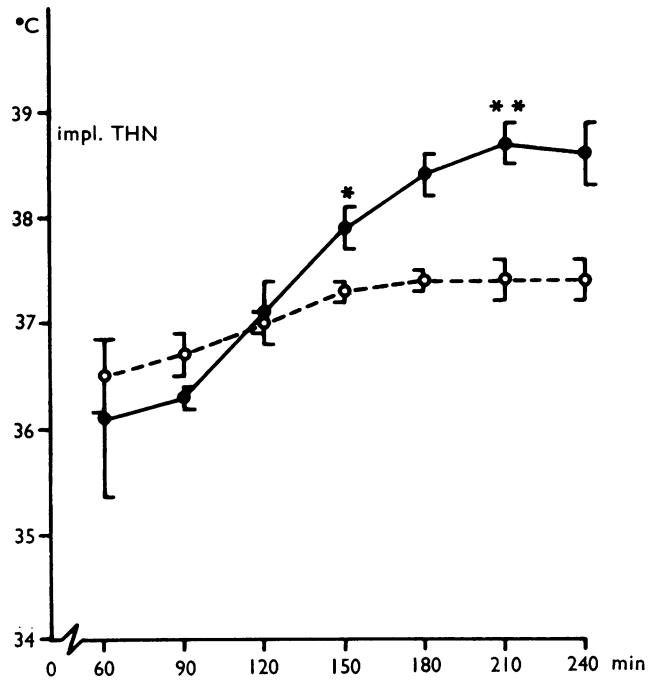

FIG. 3. Rectal temperature of unanaesthetized rats. At zero point implantation into the medial preoptic area of a cannula and injection of about 20 μg THN (continuous line). Implantation of an empty cannula (interrupted line). Each value is the mean of six observations and the standard errors are indicated. The points marked with asterisks are significantly different from the controls (* $P<0{\cdot}01$, ** $P<0{\cdot}001$).

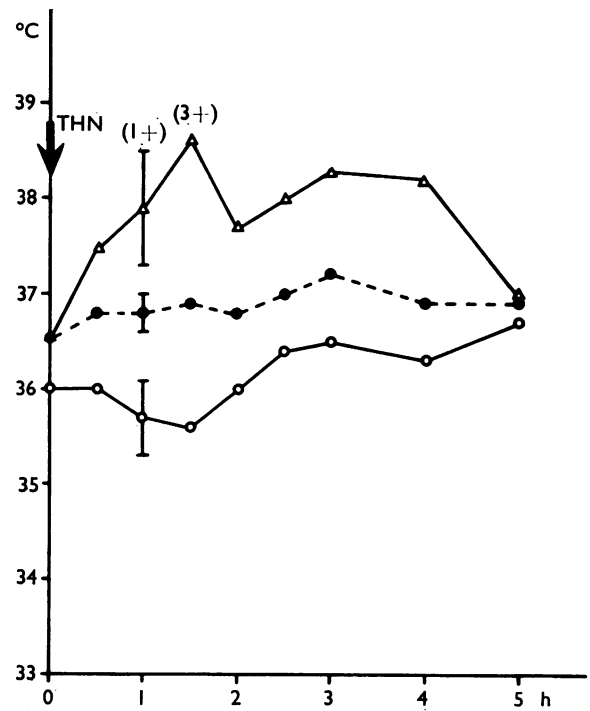

FIG. 4. Rectal temperature of unanaesthetized rats. Effect of pretreatment with nialamide (100 mg/kg i.p.) on the response to THN (5 and 40 mg/kg i.p.) given 16 h later. Each value is the mean of four observations and the standard errors are indicated. +, Number of rats that died during hyperthermia. (●– –●), Nialamide-saline. (○——○), Nialamide-THN (5 mg/kg). (△——△), Nialamide-THN (40 mg/kg).

Results

Intraperitoneal injections of graded doses of THN resulted in a dose dependent decrease in body temperature (Fig. 1). The threshold dose was 5 mg/kg and the greatest fall in temperature was obtained with 20 mg/kg. Hypothermia was maximal 1 h after the injection and temperature returned to normal in the next 3 hours.

Intracisternal injections of THN lowered body temperature in much smaller doses than an intraperitoneal injection, and the hypothermia was maximal within 10 minutes. This time interval was therefore used to determine the dose-response relationship as given in Fig. 2. The smallest dose which caused a significant decrease was 16 μg/rat ($P<0{\cdot}01$).

Implantation of crystalline THN (ca. 20 μg) caudal to the striatum and rostral to the medial preoptic area did not cause a fall but resulted in a rise of body temperature which began 2·5 h after the implantation (Fig. 3) and reached its maximum in 1 h ($P<0{\cdot}01$ and $P<0{\cdot}001$ respectively). Implantation of THN more rostral to the medial preoptic area did not affect temperature.

Effect of nialamide

An intraperitoneal injection of nialamide (100 mg/kg) had no definite effect by itself on body temperature, nor did it affect the small hypothermic response produced by THN (5 mg/kg) injected intraperitoneally 16 h later. On the other

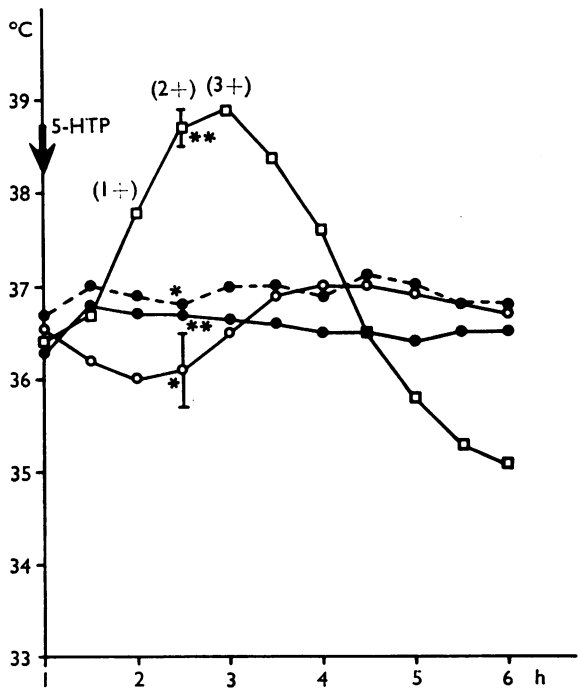

FIG. 5. Rectal temperature of unanaesthetized rats. Effect of pretreatment with nialamide (100 mg/kg i.p.) on the response to 5-HTP (60 mg/kg i.p.) given 16 h later. Each value is the mean of four observations and the standard errors are indicated. +, Number of rats that died during hyperthermia. (●– –●), Saline-saline. (○——○), Saline-5-HTP. (●——●), Nialamide-saline. (□——□), Nialamide-5-HTP.

hand, the pronounced hypothermia produced by THN (40 mg/kg) was converted into a strong hyperthermia by the pretreatment with nialamide. The results are shown in Fig. 4. During the development of the hyperthermia three out of five rats died. A similar conversion of a hypo- into a hyperthermic response by nialamide was obtained for 5-HTP. Figure 5 shows that an intraperitoneal injection of 5-HTP (60 mg/kg) lowered temperature by about half a degree, but when given 16 h after the nialamide it caused hyperthermia; temperature rose about 2° C during the following 3 h and then fell in the next 3 h to about 2° C below the preinjection level. Again, three out of five rats died during the hyperthermia.

Effect of PCPA

Pretreatment with this inhibitor of tryptophan hydroxylase enhanced the hypothermic response to THN. The pretreatment consisted of daily administration of PCPA (316 mg/kg) by stomach tube for 3 consecutive days followed a day later by the intraperitoneal injection of THN (40 mg/kg). Figure 6 also shows that the PCPA treatment by itself did not affect temperature.

Combined effect of Ro-4-1284 and DEDTC

As shown in Fig. 7 an intraperitoneal injection of Ro-4-1284 (10 mg/kg) followed 30 min later by an intraperitoneal injection of DEDTC (360 mg/kg) caused

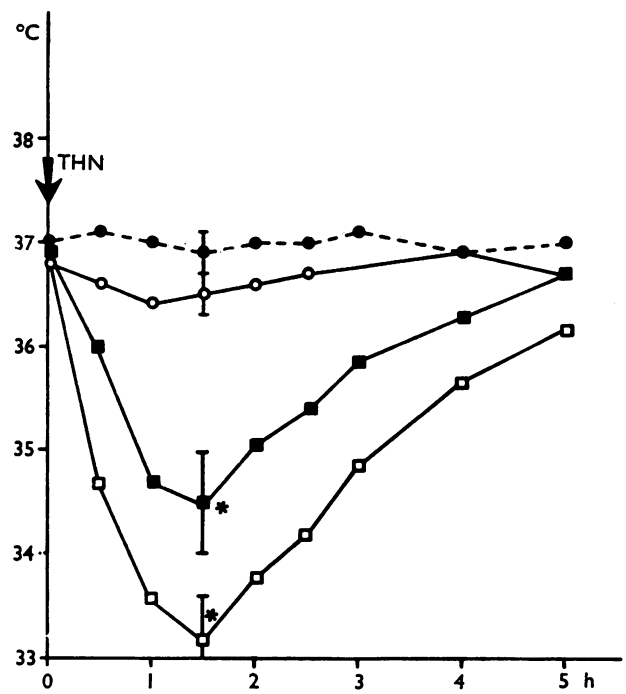

FIG. 6. Rectal temperature of unanaesthetized rats. Effect of pretreatment with PCPA (316 mg/kg p.o.) given on each of 3 consecutive days on the response to THN (40 mg/kg i.p.) given 1 day after the last PCPA injection. Each value is the mean of six observations and the standard errors are indicated. The points marked with an asterisk are significantly different from their respective controls ($P=0{\cdot}05$). (●– –●), Saline-saline. (○——○), PCPA-saline. (■——■), Saline-THN. (□——□), PCPA-THN.

a pronounced long lasting fall in temperature, but this fall was prevented, or greatly delayed and attenuated, when again 30 min later THN (40 mg/kg) was injected intraperitoneally.

Discussion

The finding that intraperitoneal administration of graded doses of THN to rats resulted in a dose dependent decrease in body temperature is in agreement with results reported by Giaja & Dimitrijevic (1933). The ability of intracisternal injections of small doses of THN, which were inactive after systemic administration, to decrease body temperature, indicates that THN acts on thermosensitive structures in the brain. Although intracisternal injection of THN causes a decrease in body temperature, implantation of the crystalline substance rostral to the medial preoptic area (MPOA) results in an increase in body temperature. The site where THN was implanted is comparable to the point of application of THN in rabbits as described by Sacharoff (1909) and by Barbour & Wing (1913), viz. the 'heat centre'. The failure of THN to provoke hyperthermia after intracisternal administration might be explained by the inability of the THN to reach these thermosensitive structures. The same may apply to harmaline which produces hypothermia without tremor on cisternal injection, but hypothermia with tremor on systemic administration (Bruinvels, 1969). These findings suggest that

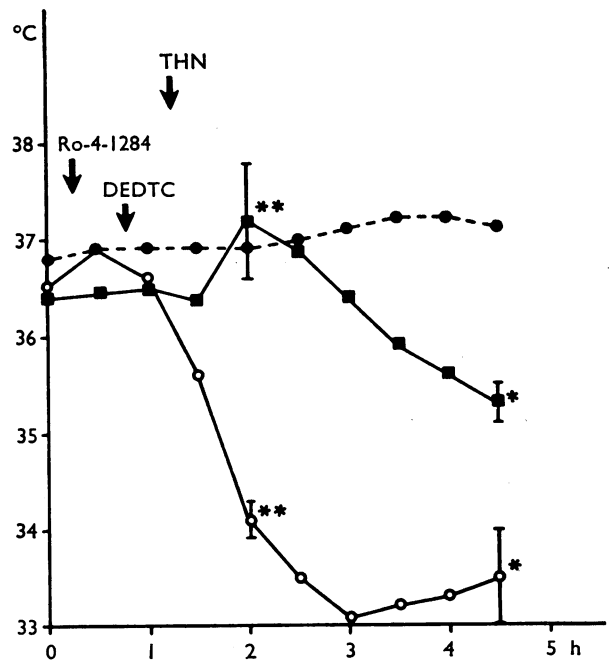

FIG. 7. Rectal temperature of unanaesthetized rats. Effect of pretreatment with Ro-4-1284 (10 mg/kg i.p.) and DEDTC (400 mg/kg i.p.) on the response to THN (40 mg/kg i.p.) given 1 h after the Ro-4-1284 and 0·5 h after the DEDTC injection. Each value is the mean of six observations and the standard errors are indicated. (●– –●, Saline-saline-saline. (○——○), Ro-4-1284-DEDTC-saline. (■——■), Ro-4-1284-DEDTC-THN. Points marked with a single asterisk are different ($P<0·01$) from each other. The significance of the difference between points marked with a double asterisk is: $P<0·001$.

drugs injected into the cisterna magna are not able to act on brain structures located anterior and dorsal to the hypothalamus.

According to Feldberg & Myers (1963) body temperature is regulated by the balance of NA and 5-HT in the hypothalamus, and recently it was shown that THN affects these monoamines in the rat brain. It inhibits the reuptake of NA and 5-HT and releases NA from its stores (Bruinvels, 1971). It is therefore suggested that the THN-induced temperature effects are mediated by NA and 5-HT, and several of the observations described can be explained in this way. For instance the conversion of the THN-induced hypothermia into hyperthermia after pretreatment of the rats with a MAO inhibitor or after depletion of monoamines with Ro-4-1284 in combination with inhibition of NA biosynthesis, suggests involvement of the monoamines in these temperature effects. Further, the combined pretreatment of monoamine depletion and inhibition of NA biosynthesis, which prevented the THN-induced hypothermia, indicates that NA is a prerequisite for the THN-induced hypothermia. The availability of NA is also important for the harmaline-induced hypothermia (Bruinvels & Sourkes, 1968) and involvement of NA in the THN-induced hypothermia would explain why a small dose of THN (5 mg/kg i.p.) did not reverse the hypothermia into hyperthermia in rats pretreated with a MAO inhibitor. This dose of THN was shown to be too small to inhibit the reuptake of 5-HT in monoaminergic fibres, but was sufficient to inhibit the reuptake of NA (Bruinvels, 1971).

On the other hand, the hyperthermia obtained with larger doses of THN could be the result of a potentiation of 5-HT at thermosensitive receptors situated rostral to the MPOA. Further evidence in favour of an involvement of 5-HT in the THN-induced hyperthermia is the hyperthermic effect of 5-HTP which, like that of THN, was obtained after pretreatment with a MAO inhibitor, and which, according to Mantegazza (1964) is prevented by the two 5-HT antagonists methysergide and cyproheptadine. Finally, the enhanced hypothermia obtained with THN in rats pretreated with PCPA, a drug which selectively depletes 5-HT in rat brain (Koe & Weissman, 1966), suggests that 5-HT counteracts the THN-induced hypothermia and thus, that 5-HT is involved in the hyperthermia. However, there is no direct evidence for a hyperthermic effect of 5-HT in rats. Injected intraventricularly (Feldberg & Lotti, 1967; Myers & Yaksh, 1968) or intracisternally (Bruinvels, 1970) it lowers temperature. This effect, however, has been ascribed to a non-specific action since imipramine did not potentiate the hypothermia produced by intracisternal injection of 5-HT (Bruinvels, 1970). Results obtained by Sulser & Sanders-Bush (1970) with reserpine after intraventricular injections of ^{14}C 5-HT and ^{14}C 5-HTP are also in favour of an unspecific action of 5-HT. After the injection of ^{14}C 5-HT, reserpine did not deplete ^{14}C 5-HT in the brain, but after the injection of ^{14}C 5-HTP depletion of its product, ^{14}C 5-HT, occurred with reserpine.

In conclusion, these results suggest that THN affects body temperature in rats by two different central mechanisms: one which is mediated by NA, probably in the hypothalamus, and which results in hypothermia, and the other, which is mediated by 5-HT, in a region rostral to the MPOA, and which results in hyperthermia.

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