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Pharmacological Properties of Tetrahydronorharmane (Tryptoline)

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Summary. Earlier in vitro experiments led to the hypothesis that tetrahydronorharmane (THN) modulates the effect of serotonin. This assumption has now been tested in vivo on rats. In addition dopaminergic mechanisms were investigated. Findings in favour of a serotonergic action of THN were:

1. The analgesic effect of THN which was antagonized by 9,10-dihydro, 10-(1-methyl-4-piperidyliden)-9-anthrol, a serotonin-receptor antagonist,
2. the hypothermic effect of THN,
3. the appetite-reducing effect of long-term treatment with THN.

An antidopaminergic effect of THN was suggested by:

1. The motility-reducing effect of THN,
2. the antagonistic effect against apomorphine-induced licking movements of acute and chronic treatment with THN and the development of supersensitivity to apomorphine one week after withdrawal of THN,
3. the antagonistic effect of THN against the contralateral turning response to apomorphine in animals with unilateral medial forebrain bundle lesions.

The antidopaminergic effects were elicited by lower doses of THN than the stimulation of serotonergic mechanisms.

The observed prolongation of hexobarbital sleeping time might be an unspecific effect. A reduction of the elimination rate of the barbiturate was not detected.

Key words: Tetrahydronorharmane – Tryptoline – β-Carboline – Serotonin – Dopamine.

INTRODUCTION

Several authors have shown a non-enzymatic reaction of tryptamine and serotonin with formaldehyde. The products formed are β-carbolines and were named tryptolines by Barchas et al. (1974). In addition a formaldehyde-producing enzyme was demonstrated in chicken, rat, and man with a maximal turnover rate at pH 6.4 and 37° C (Barchas et al., 1974; Mandel et al., 1974; Hsu and Mandel, 1975; Lauwers et al., 1975; Rommelspacher et al., 1976a). Although, there is no evidence that β-carbolines exist in vivo, this cannot be ruled out. In an attempt to assess whether the β-carboline formed from tryptamine is effective in a biological system, a competitive inhibition of the uptake of serotonin into synaptosomes prepared from the rat forebrain was found with a Ki of 6×10^{-6} M (Rommelspacher et al., 1976b). The inhibitory effect on noradrenaline uptake was low and on dopamine uptake about one order of magnitude weaker than for serotonin (unpublished results). The hypothesis was put forward that tetrahydronorharmane (THN) increases the effect of serotonin at the receptor by inhibiting the uptake of serotonin through the membrane of the nerve ending.

In this paper the effect of THN on pain reactions, body temperature, motility, hexobarbital sleeping time, food and water consumption, body weight as well as the antagonism to apomorphine has been investigated.

MATERIALS AND METHODS

Female Wistar rats (160–200 g) were kept under conventional conditions and had free access to food (standard chow, Altromin, Lage/West Germany) and water. All drugs were dissolved in saline and asministered by intraperitoneal injection if not stated otherwise.

The following substances were used: hexobarbital-sodium (Bayer, Leverkusen); apomorphine (ICN Pharmaceuticals, Eschwege). 1,2,3,4-tetrahydronorharmane (tryptoline, THN) was synthesized from tryptamine as described by Vejdelek et al. (1961). The identity and purity of the substance were ascertained by the melting point at 287–288° C and the identity of the infrared spectrum and TLC with authentic THN (Rommelspacher et al., 1976a).

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Analgesia

a) Acetic Acid Test. Experiments were performed between 10 and 12 a. m. A 10% (V/V) solution of acetic acid was prepared freshly. 0.1 ml was injected intraperitoneally 15 min after THN. The number of writhing movements was counted from the 15th to the 25th min after injection of acetic acid.

b) Tail Flick Test. Rats were placed individually into a plastic tube with an inner diameter of 6 cm and adapted for 20 min. Then the root of the tail was heated with a focussed lamp (halogen-dellaphot 100 W, Osram). The distance between the tail and the bulb was 4.5 cm. The time from the beginning of the exposure to a distinct reaction of the animal was measured. Jerking or screaming of the animals was regarded as a distinct reaction. The strength of the heating-lamp was adjusted by a rheostat so that controls responded to the heat after about 5 s. No animal was exposed to the heat for longer than 10 s.

Body Temperature

Body temperature was measured with a thermistor probe (type TE 3, ELLAB, Copenhagen) which was inserted 3 cm into the rectum. As described for analgesia tests the experiments were performed between 10 and 12 a. m.

Motility

The experiments were performed during the active phase of the rats between 8 and 10 p. m. Groups of 3 animals were placed into plastic cages (22 × 39 × 15.5 cm) which were divided by 2 straight lines into 4 equal parts. The number of crossings of the lines was counted for each animal from the 15th to the 30th min after treatment with THN or saline.

Hexobarbital Sleeping Time

The experiments were carried out in a quiet room at 22° C. Ten minutes before and, in another series of experiments, 30 min after the injection of 100 mg/kg hexobarbital i. p. various doses of THN were injected into the other side of the abdominal cavity. The time from the injection of hexobarbital to the regaining of the righting reflexes was regarded as sleeping time.

Hexobarbital Concentration in Blood and Brain

Animals were decapitated at different times after the injection of 100 mg/kg hexobarbital or the combined treatment with hexobarbital and THN. Their blood was collected in a test tube containing heparin. The brains were quickly removed from the skull and homogenized in ice-cold phosphate buffer (0.067 M, pH 7.7). Hexobarbital contents of brain and blood were determined according to the method of Brodie et al. (1953) as modified by Remmer (1959).

Food and Water Intake and Change of Body Weight

Three rats were housed in a familiar cage (22 × 39 × 15.5 cm) and received dry food (Altromin) and a sucrose solution (40 g/l) dissolved in tap water which contained THN in various concentrations and sucrose solution without THN (controls), respectively, ad libitum. Body weight, food intake and consumption of the sucrose solution were recorded daily at 10 a.m. The ingested food and fluid were recorded by weighing the residue and then replaced.

The THN solution tasted somewhat bitter. To exclude that the observed reduction of fluid consumption was caused by the unpleasant taste, 3 groups of 3 rats each received quinine (0.02%) dissolved in sucrose solution (40 g/l) over a period of 20 days. The fluid consumption was recorded as described above for THN-treated rats.

Apomorphine Antagonism

a) Stereotyped Behaviour. After injection of various doses of apomorphine the rats were placed individually into a plastic tube (6 cm inner diameter). The compulsive licking movements were counted for 30 s every 10 min. The observation period was 60 min.

b) Rotational Behaviour. Medial forebrain bundle lesions were placed under chloralhydrate anaesthesia as described earlier (Walters et al., 1973; Rommelspacher and Kuhar, 1975). The head of the animal was mounted in a stereotaxic apparatus (David Kopf, Instruments). The skull was opened in the appropriate position with a dental drill. Radiofrequency lesions (500 KHz) were made with a lesion generator (Radionics, Inc. Burlington, U.S.A., Model RFG-4, 60° C for 10 s.) at the following coordinates according to König and Klippel (1967): Anterior A 5150, horizontal 1.2 mm, vertical 8.2 mm. The nigro-striatal tract of the right side was interrupted by lesioning the medial forebrain bundle at the level of the rostral end of the supramammillary decussation. The experiments were performed 2 weeks to 3 months after placement of the lesions. Only rats turning 360° at least twice a minute were included in the test. The animals were reused in each experimental session but at least 1 week elapsed between 2 experiments. The rats were injected i. p. with THN followed immediately by 4 mg/kg apomorphine s. c. 15 min later rotations were counted for 1 min every 15 min. The observation period was 90 min. The midbrains of the rats were sectioned and stained (cresyl violet) to verify histologically the site of the lesion.

Statistics. The statistical significance of the results was determined by Student's *t*-test. The regression lines were calculated by the method of least squares. The median lethal dose (LD_{50}) was determined as described by Spearman and Kärber (Cavalli-Sforza, 1969).

RESULTS

Five groups of 10 rats each were used to determine the LD_{50} of THN. 98 ± 9 mg/kg was calculated for i.p. injection.

Analgesia

An analgesic effect was found in 2 tests. Figure 1 shows the dose-response curves for the acetic acid-induced writhing and the tail flick test. It seemed to be necessary to use two different methods because rats treated with high doses ($\gtrsim$ 50 mg/kg) screamed but did not resist or flee, when their trunks were touched gently in an open field situation.

Body Temperature

A dose-related hypothermia was found after administration of 6.25 – 50 mg/kg THN. Doses above 6.25 mg/kg reduced body temperature significantly. In Figure 2 the time course of hypothermia is shown after injection of 50 mg/kg THN. The maximum effect appeared after 90 min and disappeared with a "half time" of 90 min.

Motility

In the motility test there was a dose-dependent effect of THN between 3 mg/kg and 12.5 mg/kg which elicited the maximal effect (Fig. 3).

Hexobarbital Sleeping Time

THN prolonged hexobarbital sleeping time in a dose-dependent manner when administered either 10 min prior or 30 min after hexobarbital. As shown in Figure 4 no difference of the maximal effect was found between the 2 regimens of injection.

The hexobarbital sleeping time may be prolonged by an inhibition of metabolizing enzymes in the liver and also by a cellular mechanism in the CNS. Since the "half time" of hexobarbital in blood and brain was not altered by THN (Fig. 5) functional effects in the CNS may be considered to be responsible for the action of THN.

Effect of THN on Food and Water Intake and the Alteration of the Weight Gain

Rats received THN dissolved in sucrose and dry food ad libitum. Controls drank sucrose solution. The intake of sucrose solution by control rats (54.8 ml/100 g) was almost three times as high as the usual intake of tap water (19 ml/100 g) during the first 24 h. At the following days the fluid-consumption declined and reached values of water-treated animals at the fiftieth day (Fig. 6, Magour et al., 1976). After an initial phase during which the animals treated with the solution of THN drank less than half of controls, the rats started to increase their water intake. Even at a concentration of 0.015% they never ingested as much as controls. At higher doses some of the animals died (2 animals out of 12 at 0.06%, 4 animals out of 12 at 0.12% THN).

To test how far the bitter taste of the THN solution may have influenced fluid consumption 9 rats were treated over a period of 20 days with quinine in the same way as described above for THN-treated animals. 18.7 ml/100 g was calculated as the average amount of fluid intake per day of the quinine-treated rats which is close to the consumption of animals drinking tap water.

A relative decrease of the ingestion of the drug results if animals drink less from highly concentrated fluids. We therefore calculated the amount of THN consumed by the different groups. Two phases of daily THN intake were observed. During the first 4–7 days an almost parallel increase occurred at all concentrations. Then, after a maximum was reached, the amount of THN consumed daily did not increase

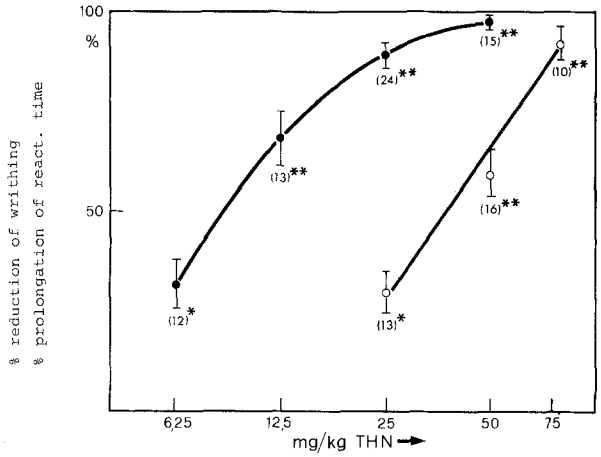

Fig. 1. Dose-response curves for the reduction of acetic acid-induced writhings (controls: 13.4 ± 1.4/10 min; $n = 54$), (●——●) and the prolongation of reaction time of tail flick (○——○) (reaction time of controls: 5.0 ± 0.2 s; $n = 22$) after i. p. injection of THN. Each circle represents the mean, vertical bars S.E.M. Numbers of animals are given in parenthesis. Differences from control values: * $p < 0.01$; ** $p < 0.001$

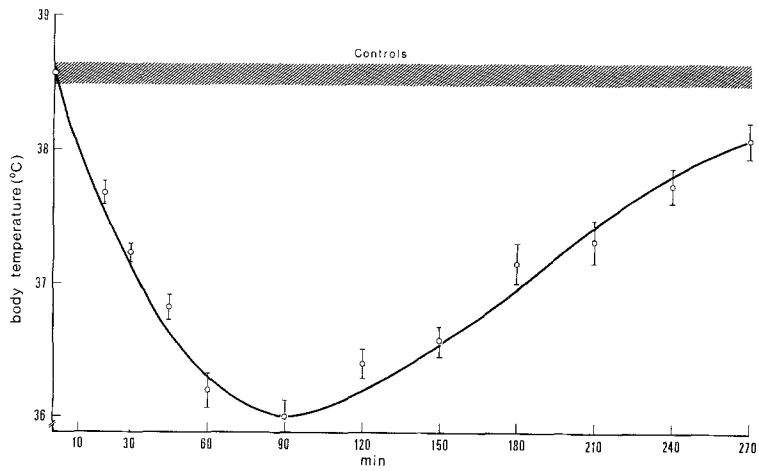

Fig. 2. Time course for the hypothermic effect of 50 mg/kg THN. Each circle represents the mean from 10 rats, vertical bars indicate S.E.M. The hatched area shows the mean ± S.E.M. for 10 controls

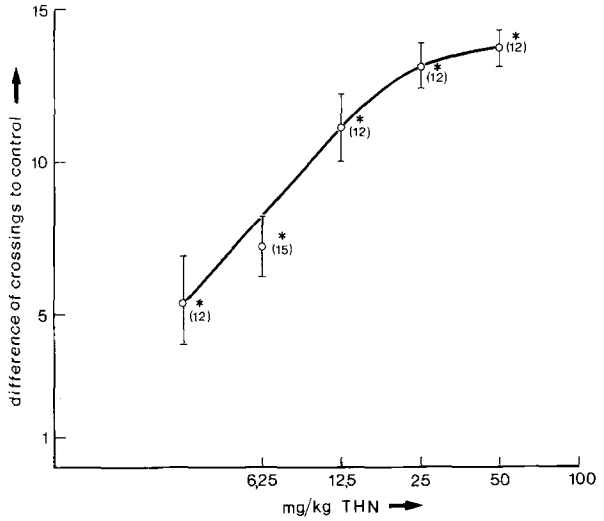

Fig. 3. Dose-response curve for motility changes after i.p. injection of THN. Each circle represents the mean from 12–15 rats, vertical bars indicate S.E.M. Controls: 17.4 ± 1.4 (n = 18). Differences from control values: * $p < 0.01$

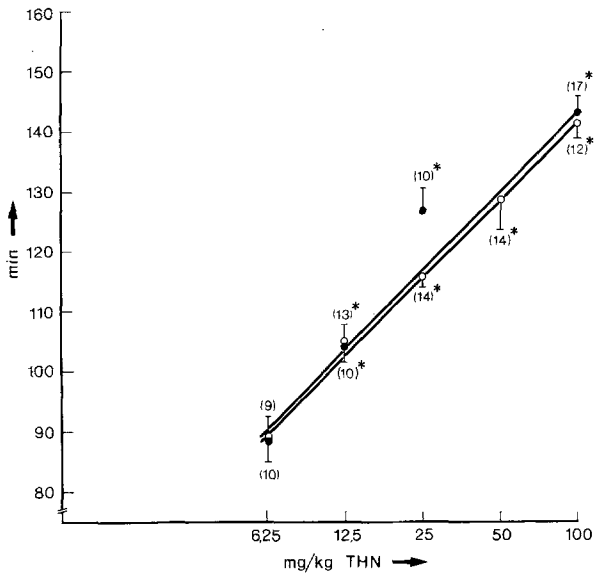

Fig. 4. Dose-response curve of THN on hexobarbital sleeping time. THN injected 10 min prior to (O———O) or 30 min after (●----●) 100 mg/kg i. p. hexobarbital. Each point represents the mean, vertical bars the S.E.M. Numbers of animals are given in parenthesis. Sleeping time of control animals 84 ± 3 min (n = 31). Differences from control values: * $p < 0.01$

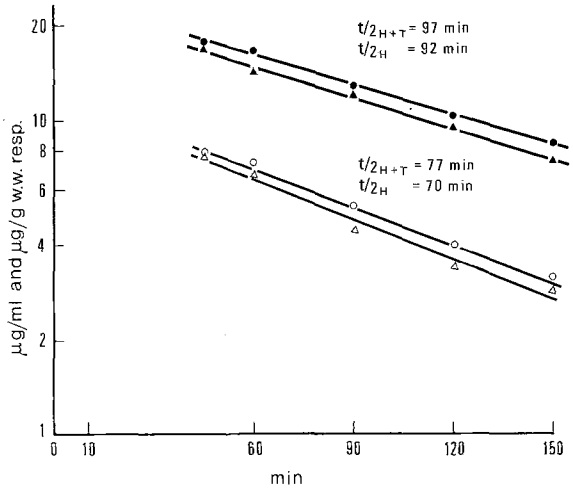

Fig. 5. Regression lines for the rate of elimination of hexobarbital from brain (lower plot) and blood plasma (upper plot). In the combined treatment THN (100 mg/kg) was injected 30 min after hexobarbital (100 mg/kg). Shown are means of at least 8 experiments each. H: Hexobarbital; $H + T$: combined treatment. (△———△ ▲———▲): hexobarbital; (O———O ●———●): combined treatment

further and was ingested constantly during the observation period. Then animals treated with a 0.015% solution received an average of 13 mg/kg and day (17), the respective values for the other groups were: 0.03% – 30 mg/kg (15 days), 0.06% – 49 mg/kg (15 days). For the 0.012% THN no maximal level was demonstrable and therefore no average value for the amount consumed daily was calculated.

During the first days of the experiments the rats ate less than controls. The effect was dose-dependent (Fig. 7).

A reduced weight gain can be deduced from these data. Animals treated with a 0.015% solution showed a reduced body weight only at the second and third day. At the medium concentration (0.03%) a deviation from controls was found up to the sixth day. The 2 other groups did not reach control values during the observation period. Noteworthy was the almost parallel course of all curves after some days of treatment. This was the case after the fifth, seventh, nineth and eleventh day, respectively, for the increasing concentrations. Thus, although the animals gained weight at the same rate as controls after this period. the gain occurred at a lower level.

Antagonism of Apomorphine-Effects by THN

a) Stereotyped Behaviour. The effect of apomorphine on dopaminergic neurones can be measured quantitatively by counting licking movements. Figure 8 shows in a threedimensional plot the time-course and the dose-dependence of the licking movements after apomorphine (a) and the alterations of these relations by simultaneous treatment with THN (b). The maximal response to apomorphine was reached at 1 mg/kg and a further increase of the dose led to a prolongation of the effect. THN in doses of 25 and 50 mg/kg antagonized the effect of apomorphine as shown in Figure 8b.

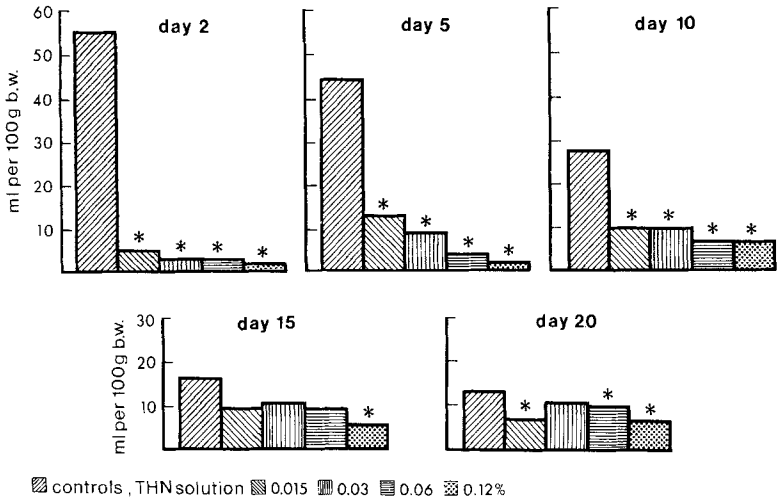

Fig. 6. Effect of THN on fluid intake in 5 groups of 12 rats each. Controls: sucrose solution (40 g/l); treated groups: sucrose + THN: Bars represent 24 h periods. * Significantly different from controls ($p < 0.01$)

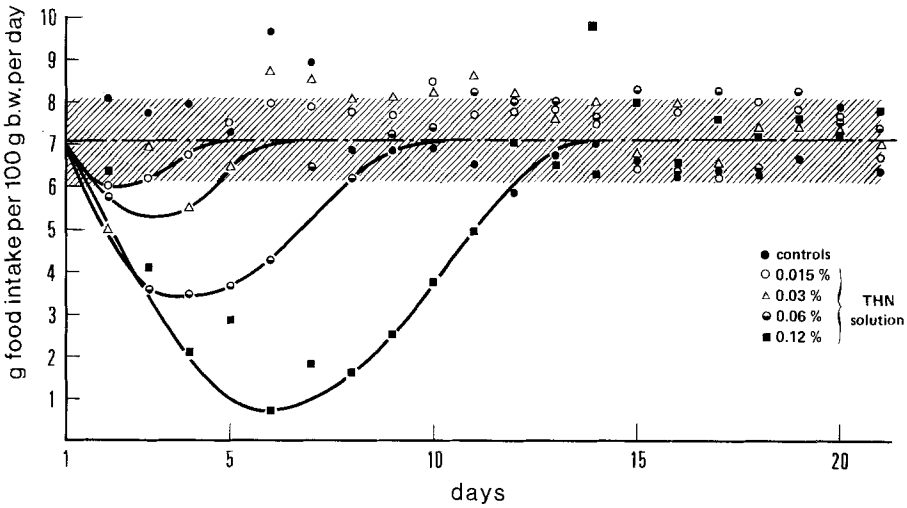

Fig. 7. Time course of the effect of THN on daily food intake in rats treated as indicated in Figure 6. The hatched area gives the mean ± S.E.M. from controls

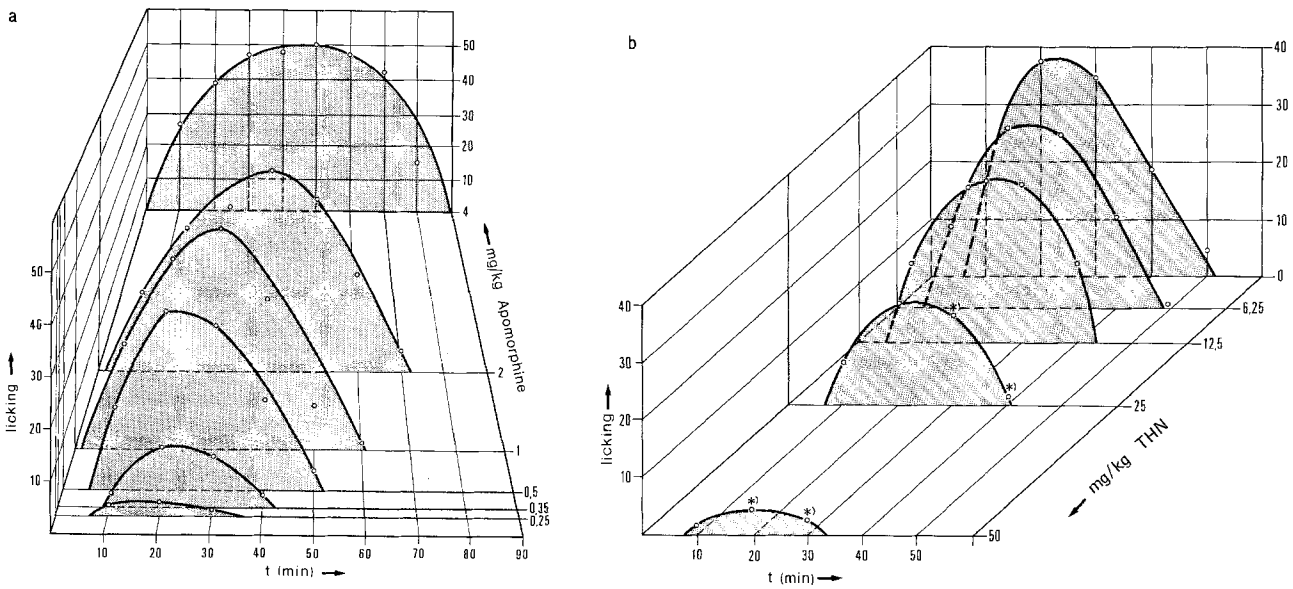

Fig. 8 a and b. Dose-response curve and time course of the effect of apomorphine and apomorphine combined with THN on the production of compulsive licking movements counted for 1 min. (a) s. c. injection of different doses of apomorphine; (b) s. c. injection of 0.5 mg/kg apomorphine combined with i. p. injection of different doses of THN. Each point represents the mean from ten rats. Differences from treatment with apomorphine alone: $*p < 0.01$

Table 1. Effect of THN on rotational behaviour

Controls	6.25 mg/kg	12.5 mg/kg	25.0 mg/kg	50.0 mg/kg
7.6 ± 2.1 (9)	3.6 ± 1.8 (9)	2.2 ± 1.4 (9)*	1.9 ± 0.8 (9)**	1.2 ± 0.5 (9)**

The numbers are the mean ± S.E.M. of turnings during the sixty first minute after injection of apomorphine (4 mg/kg s. c.) and different doses of THN i.p. Numbers of animals are shown in parentheses. Differences from control values (4 mg/kg apomorphine s. c.). $p < 0.05$; $^{**}p < 0.01$

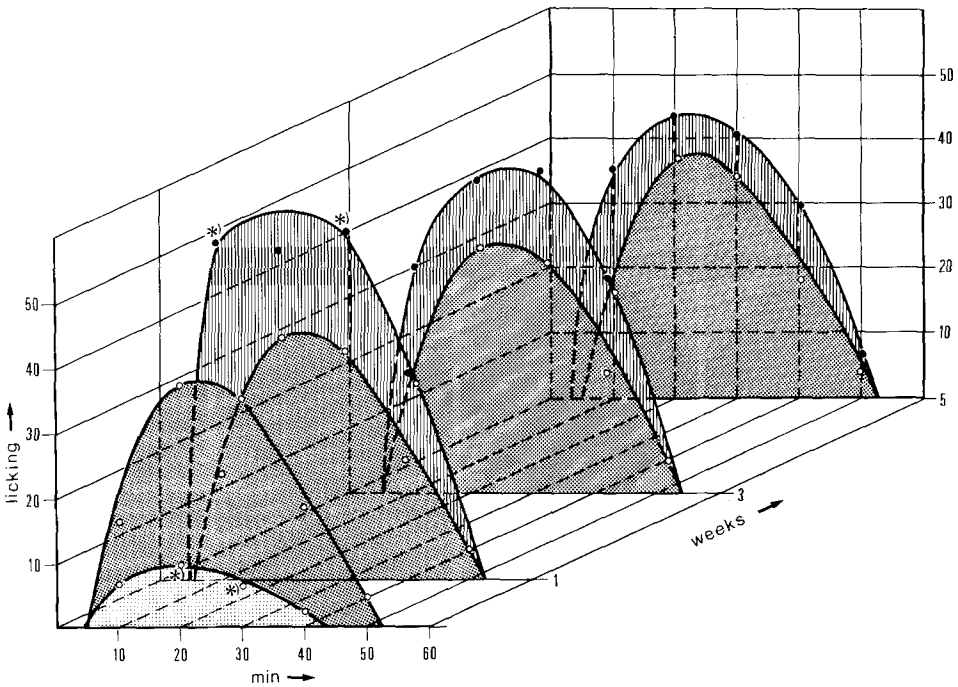

Fig. 9. Time course of treatment with 0.12% THN in the drinking water on the licking movements (counted for 1 min) elicited by 0.5 mg/kg apomorphine s. c. Immediately after withdrawal (▤), 1,3 or 5 weeks after withdrawal (▥), and the response of naive rats (▨). Each point represents the mean of eight animals. $^{*}p < 0.01$

b) Rotational Behaviour. In the medial forebrain bundle, dopaminergic neurones are located which connect substantia nigra and striatum. In response to a unilateral lesion in this tract the animal turns ipsilaterally. A few weeks later when neurones have degenerated the postsynaptic receptors are supersensitive. Then the injection of apomorphine leads to a contralateral rotation.

In our experiments, performed 2 weeks to 3 months after the stereotaxic operation, this finding has been confirmed and treatment with THN reduced the contralateral turning in a dose-dependent manner (Table 1). Two out of 9 animals turned ipsilaterally under these conditions.

c) Experiments with Animals Treated Chronically with THN. Rats treated chronically with THN received the substance as described in the paragraph dealing with food and water intake. Immediately after withdrawal of THN the animals were injected with 0.5 mg/kg apomorphine s.c. Compared to controls the number of licking movements was strongly reduced (Fig. 9). Thus, no tolerance developed to the antagonism of THN to apomorphine-induced licking. One week after termination of the chronic treatment with THN an increased licking rate was found. Five weeks after withdrawal of THN this "supersensitivity" to apomorphine had disappeared.

DISCUSSION

Studies on the high affinity uptake of serotonin had shown an interference of THN on the synaptosomal membrane with the transmitter (Rommelspacher et al., 1976a). Using a suspension of synaptosomes from mouse brain an inhibitory effect of THN and 6-methoxy-THN on serotonin uptake was found by Buckholtz and Boggan (1976). Tuomisto (1973) reported an inhibition of 5-HT uptake into synaptosomes also

by tetrahydroharmane and 6-hydroxytetrahydroharmane.

Although there is little evidence concerning the specific role of serotonin in the CNS, it has been suggested that serotonin is involved in the central control of pain, food and water intake and thermoregulation.

The assumption that serotonergic neurones regulate the perception of pain in the ZNS is based on the observation that injection of p-chlorophenylalanine (pCPA) lowers the 5-HT content in the brain and increases the sensitivity of rats to painful stimuli as measured by the flinch-jump and hot plate methods. The same effect has been observed after brain lesions which destroy serotonergic pericaria in the raphé nuclei or axons located in the medial forebrain bundle or the septal area (Tenen, 1967; Lints and Harvey, 1969; Yunger and Harvey, 1973). 75 mg/kg 5-hydroxytryptophan, which brought the content of 5-HT back to control values, reversed the effect of pCPA and lesions (Tenen, 1967; Harvey and Lints, 1971; Harvey et al., 1974).

THN was found to increase the 5-HT content in the rat forebrain (Rommelspacher et al., 1976b) and one could expect an analgesic effect, especially if one also takes into consideration that THN inhibits the uptake of serotonin into synaptosomes. The effect of THN in doses of 50 mg/kg and more first suggested a decrease of the analgetic threshold, concluded from the reaction of the rats which screamed in response to a gentle touch by the investigator. With both the acetic acid and the tail flick method, an analgesic effect of THN has been established. 50 mg/kg THN was about aequieffective with 5 mg/kg morphine. 9,10-Dihydro-10-(1-methyl-4-piperidyliden)-9-anthrol (WA 335 BS, Thomae, Biberach) a 5-HT-receptor antagonist, abolished the analgesic effect of THN (Heyck Cohnitz, unpublished observations).

The hypothermia after THN is in accordance with its serotonergic effect, since Feldberg and Lotti (1967) found a hypothermia after injection of 2–20 µg of 5-HT into the lateral ventricle of rats. It is also in agreement with the effect of 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine (Lilly 110140) which is considered a specific inhibitor of 5-HT uptake and which lowers body temperature (Miliaressis and Jacobowitz, 1976).

Intraventricular injection of 5-HT elicits a reduction of food intake, which can be blocked by cyproheptadine, a 5-HT antagonist (Kruk, 1973). Also the anorexic effect of fenfluramine as well as of Lilly 110140 is supposed to be due to a stimulation of serotonergic neurones (Blundell and Lesham, 1975). The latter drug potentiates the reduction of food-consumption found after treatment with 5-HTP even when its own effect had completely worn off (Wong et al., 1974; Goudie et al., 1976). Blundell and Lesham (1975) assume a central site of action of the uptake inhibitor. In chronic experiments THN reduced food and fluid intake as well as body weight. Tolerance developped to the depressant effect of THN on food intake. Fluid consumption reached control values only when low doses of THN were offered. Whether these effects are produced by a serotoninergic action or whether an antidopaminergic action of THN plays an additional role remains uncertain, because also pimozide, the dopamine-antagonist, reduced water intake (Nielsen and Lyon, 1973). Involvement of dopaminergic neurones in the control mechanism of food and water intake, however, is still controversial. Apomorphine (0.1–1.0 mg/kg) produced a "dose-dependent" inhibition of drinking (Janssen et al., 1967; Nielsen and Lyon, 1973). Lesions of the substantia nigra pars compacta generated a severe aphagia and adipsia (Ungerstedt, 1971a and b). On the other hand, intrahypothalamic injections of dopamine failed to alter either feeding or drinking (Leibowitz, 1974). As a third explanation the somewhat bitter taste of the THN solution was considered as a cause of the reduction of food and water intake. To exclude this possibility we dissolved quinine in the sucrose solution so that it tasted to the investigator like the 0.12% THN solution. Quinine compensated the increased fluid intake found in sucrose-treated animals but did not reduce it below that in animals drinking tap water.

The analgesic action. the hypothermia and the reduction of food and water intake support the hypothesis that THN has a serotoninergic effect.

The motility of rats was reduced by THN injection. This finding might be caused by serotonergic neurones, too, because a state of general excitement with increased locomotor activity was observed after selective reduction by p-CPA or midbrain raphélesions of brain 5-HT (Kostowski et al., 1968; Sheard, 1969; Brody, 1970). On the other hand, injection of precursors of serotonin produced a special behavioural syndrome with hyperactivity (for review see Jacobs, 1976). The hyperactivity was reduced by p-CPA (Grahame-Smith, 1971). Foldes and Costa (1975), however, found no correlation between motility and 5-HT accumulation rates, when an inhibitor of monoamine oxidase was injected. They concluded that hypermotility, elicited by indoles, could be triggered by a substance other than 5-HT acting on catecholaminergic neurones.

The prolongation of hexobarbital sleeping time by THN seems to be unspecific because this effect is seen after many drugs, e. g. pyridin-nucleotides, neuroleptics and tetrahydrocannabinol (Coper et al., 1968; Rating et al., 1972; Jend and Coper, 1974). Noteworthy was the finding that the sequence of the injec-

tions of THN and hexobarbital played a minor role. One explanation could be a slow elimination rate for THN as found for the structurally related harmaline (Zetler et al., 1972).

Apomorphine is supposed to stimulate dopamine receptors directly to produce stereotyped behaviour in rats (Ernst and Smelik, 1966; Andén et al., 1967; Ernst, 1967). In our experiments apomorphine was tested on licking movements. Dopamine-antagonists like neuroleptic drugs inhibit the licking movements (Janssen et al., 1960; 1967). THN had the same effect after acute and chronic application.

On the other hand an increased sensitivity to apomorphine of the same animals was found 1 week after withdrawal of THN. Two weeks later this effect has disappeared, though statistical evidence for the disappearance of supersensitivity was not obtained. The "supersensitivity" resembles that which had been observed after chronic application of clozapine and after haloperidol (Smith and Davis, 1976), two dopamine antagonists.

Drug effects on central dopaminergic neurones can be studied using the pathway from substantia nigra (SN) to neostriatum. Electrical stimulation of the SN of one side produces a contralateral turning response (Arbuthnott and Crow, 1971). Unilateral axotomy leads to ipsilateral rotation after injection of putative dopamine receptor antagonists, whereas the opposite behaviour occurs after agonists like apomorphine. The circling can be explained on the basis of an imbalance between the 2 dopaminergic tracts (Andén et al., 1966; Ungerstedt, 1971a,b; Costall et al., 1972). THN blocked the effect of the dopamine-like agonist and in some cases even reversed the response since ipsilateral turning was observed.

The decreased motility in THN-treated rats may be caused by an antidopaminergic action, too, since drugs which reduce central catecholamine transmission produced behavioural states of sedation and inactivity (v. Brücke et al., 1969).

In all tests which presumably involve dopaminergic mechanisms THN exerted its action at relatively low doses compared to the other experiments. This holds true for motility, licking movements and rotational behaviour.

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