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Chronic Methylxanthine Treatment in Rats: A Comparison of Wistar and Fischer 344 Strains

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LLOYD, H. G. E. AND T. W. STONE. *Chronic methylxanthine treatment in rats: A comparison of Wistar and Fischer 344 strains.* PHARMAC. BIOCHEM. BEHAV. **14**(6) 827–830, 1981.—Caffeine, theophylline or aminophylline were administered chronically to rats of both sexes, in the weight range 30–245 g. Self-injurious behaviour was noted only rarely in Wistar rats allowed free access to food, but developed over 3 to 4 weeks in half of the animals given a restricted diet of about one third of the intake of control rats. Fischer rats showed self-injurious behaviour more readily, 87% of animals showing signs within 9 days even on an ad lib diet. It is suggested that Fischer rats treated with methylxanthines may provide a model of the Lesch-Nyhan syndrome. Behavioural observations made during the period of methylxanthine treatment suggest that an activation of both the dopamine and 5-hydroxytryptamine neurone systems may be produced. Further work will seek a relationship between these systems and self-injurious behaviour.

Methylxanthines Caffeine Theophylline Purines Self-injury Lesch-Nyhan syndrome

A DEFICIENCY of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT), one of the purine salvage enzymes which helps conserve cellular purines otherwise destined for excretion, can cause the Lesch-Nyhan syndrome [9]. This condition, affecting male children, is characterised by involuntary movements, mental retardation and, in some cases a self-injuring compulsion most commonly seen as a destruction of the lips and fingers by biting [9].

Shortly after the original description of the human syndrome it was reported that the administration of caffeine to rats could also induce the appearance of self-injury behaviour, seen particularly as a biting of the forepaws [8]. It has subsequently become clear that methylxanthines, which include caffeine (1, 3, 7-trimethylxanthine) and theophylline (1, 3-dimethylxanthine) increase the release and turnover of amine transmitters in the central nervous system [1,13] and the possibility arises that such changes of amine function may play a causative role in the behavioural signs. The present study was therefore designed to investigate more closely the behavioural effects of methylxanthines in rats, paying particular attention to the incidence of amine-related behaviour patterns.

METHOD

Rats were of either Wistar or Fischer 344 strains as noted in Results.

Acute Treatment

In order to determine which behavioural components might be attributable to a common action of methylxanthines and derivatives rather than to a unique action of an individual drug, 6 different xanthines were administered to animals acutely at doses of 0.5 and 1 mmol, kg^{-1}, SC. The following xanthine derivatives were used; caffeine, theophylline, aminophylline, acepifylline (Delandale Labs), diprophylline (Nord) and oxyphylline (Astra). After a period of 30 to 45 minutes, the animals were observed for at least 15 minutes and the occurrence of various behaviours noted.

Chronic Treatment

For chronic treatment, the drugs used were caffeine, theophylline and aminophylline (Sigma). For both IP and oral administration the drugs were dissolved in saline with warming or suspended in saline. Drugs were administered in a volume of 5 ml/kg IP or 10 ml/kg orally. Control animals received the saline vehicle alone.

Initially groups of 4 male rats were treated with increasing doses of caffeine or aminophylline, SC (up to 150 mg/kg/day), caffeine orally (up to 185 mg/kg/day), aminophylline orally (up to 346 mg/kg/day) or theophylline orally (up to 325 mg/kg/day). Treatment was continued over periods of three weeks. Subsequently larger groups of rats

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were tested with caffeine, 185 mg/kg/day orally (7 male, 7 female) or aminophylline, 300 mg/kg/day orally (14 male, 9 female) for 4 weeks.

At the time of every drug administration, each animal was examined for signs of self-injury behaviour. Approximately 10 to 15 minutes after drug administration, animals were observed for 2 minutes while in their home cages. A number of these animals were also observed in a novel open-field environment formed by a cylindrical perspex pen of diameter 0.66 m. These observations were made for a 15 minute period, between 30 and 60 minutes after dosing by an experimenter unaware of the treatment status of the animal.

In these experiments the emphasis is on the presence or absence of different behavioural components, and we have attempted to note a wide range of behaviours (Table 2). In doing so, however, we have not at this stage attempted to quantify the behaviours, in any detail, and the results have been expressed simply as the percentage of animals exhibiting a particular behaviour.

The terms used in the tables relate to the following behaviours:

(1) Self-injury behaviour (s.i.b.): Self-injurious behaviour was manifested in several ways, ranging from the plucking of fur from the body, particularly the abdomen or throat, through to severe biting of the tail or paws, usually the forepaws. Fur-plucking was clearly distinguishable, as a heavy localized loss of fur, from the generalised thinning of the fur previously reported to accompany methylxanthine administration. As soon as an animal was found to show such symptoms, the extent of injury was noted, and the animal killed.

(2) Paw shake: simultaneous shaking of both forepaws while resting on hind-limbs.

(3) Jaw movements: apparently undirected chewing, licking or gnawing movements.

(4) Teeth chattering: audible teeth clashing.

(5) Head movement: Repetitive head movements either from side to side or up and down.

(6) Rubbing jaw on floor: flattened posture; walking slowly forward with lower jaw thrust forward and rubbing along floor.

(7) Ptosis: eyes at least approximately half-closed compared with controls.

(8) Straub tail: tail stiff, held vertically spontaneously. (The tail was not stroked into this position.)

(9) Backward walking: at least two clear full paces backwards with all four paws. Often accompanied by a tendency to circle.

(10) Jumping: All four paws leave the floor.

(11) Squeak on touch: very light touch (no pressure) with finger or object (pencil) causes vocalization.

(12) Treading: kneading movement; alternate lifting of forepaws.

(13) Limb abducting: splaying of hindlimbs.

RESULTS

Acute Administration

The occurrence of various behaviours in the Wistar rats treated acutely with methylxanthines is summarised in Table 1.

TABLE 1

THE OCCURRENCE OF BEHAVIOURAL COMPONENTS IN RATS (WISTARS n=20) AFTER THE ACUTE ADMINISTRATION OF METHYLXANTHINES

Behaviour	Percent animals exhibiting
Restlessness	30
Rearing	35
Ptosis	80
Grooming	60
Jaw movements (including licking and chewing)	55
Squeak on touch	45
Teeth chattering	15
Jumping	10
Diarrhoea	10
Body shakes	15
Head shakes	30
Paw shakes	10
Backward walking	0
Rubbing lower jaw	15
Sensitivity to noise	15
Tail erection (Straub)	10

Chronic Treatment

A number of factors were examined for their influence on the response of animals to the chronic administration of methylxanthines, particularly with respect to the incidence of self-injurious behaviours.

Diet. Of the total of 60 animals receiving methylxanthines chronically only 1 male receiving caffeine 185 mg/kg/day and 1 receiving aminophylline at 300 mg/kg/day showed s.i.b. These animals were all permitted access to food ad lib, consuming 27.6±0.8 g/day (s.e.; n=60).

However, of a total of 23 males and 21 females on a reduced diet (33% of normal; range 7–12 g/day), and treated with caffeine, 185 mg/kg/day orally, 9 males and 7 females exhibited s.i.b. Of some interest here was the observation that one male control animal on the reduced diet also exhibited s.i.b.

Sex. From the previous section it will be apparent that there was little evidence of an effect of gender on the response to methylxanthines.

Age. The rats used in the above experiments ranged from an initial weight of 30 g, to 245 g in different groups. Again this factor seemed not to be related to the occurrence of s.i.b.

Strain. All the above experiments were performed on Wistar animals. Using rats of the Fischer 344 strain, however, revealed a much higher susceptibility to s.i.b. A total of 12 males and 13 females were treated with caffeine, 185 mg/kg/day orally, whilst being permitted free access to food. A majority of these animals developed s.i.b. within a relatively short space of time, often only 2 or 3 days after beginning treatment. Within 9 days of starting the caffeine administration, 20 animals (8 male, 12 female) showed s.i.b.

During the chronic administration of methylxanthines to the animals described in the sections above, more detailed behavioural observations were made in an attempt to relate the appearance of specific amine-related behaviours to the occurrence of s.i.b. These observations are summarised in Table 2.

TABLE 2

BEHAVIOURS OBSERVED DURING CHRONIC TREATMENT OF RATS WITH METHYLXANTHINES

Strain	Wistar	Wistar	Wistar	Wistar	Fischer	Fischer
Number	24	14	14	28	8	16
Drug	vehicle	caffeine	aminophylline	caffeine	vehicle	caffeine
Dose	—	185 mg/kg/day	300 mg/kg/day	185 mg/kg/day	—	185 mg/kg/day
Diet	ad lib	ad lib	ad lib	33%	ad lib	ad lib
Behaviour						
s.i.b.	4	7	7	46 (0.05)	0	87 (0.001)
grooming	12	7	0	7	36	56
paw shake	8	30	57	0	25	87 (0.05)
jaw movements	12	57	64	0	12	93 (0.005)
chattering	0	7	0	0	0	62 (0.005)
head movement	0	0	0	0	0	37 (0.05)
rubbing jaw on floor	0	93 (0.001)	93 (0.001)	18	0	0
Ptosis	4	71 (0.01)	64 (0.05)	40 (0.05)	0	44 (0.05)
Straub tail	4	14	7	4	12	31
piloerection	0	0	36	0	0	12
backward walking	0	36	30	0	0	31
rearing	54	21	57	25	50	50
jumping	4	7	14	21	0	31
squeak on touch	0	57 (0.05)	78 (0.05)	18	0	—
diarrhoea	0	0	0	0	0	6
rhinorrhoea	0	0	43 (0.05)	0	0	0
treading	0	0	0	0	0	0
limb abduction	0	0	0	0	0	0
head shake	8	0	0	0	25	81
body shake	8	50	0	0	18	6

For ease of comparison across the table, the figures show the percentage of animals exhibiting each behaviour. A chi-squared test was performed on the results, to compare each group of test animals with the corresponding control group. The levels of significance achieved (p values) are indicated in parentheses.

DISCUSSION

The catalogue of behaviours observed after the acute administration of methylxanthines is similar to that reported previously by Collier and his colleagues [5], and referred to as the quasi-morphine abstinence syndrome. Thus, among the effects most commonly produced were ptosis, jaw movements including chewing and licking, head or body shakes, and a marked sensitivity to touch reflected in the animals' vocalisation on touch or handling.

Two conclusions may be drawn from the chronic administration of the methylxanthines. Firstly, although caffeine or aminophylline, administered to normal rats, induce self-injurious behaviour (s.i.b.) only rarely, the use of rats on a restricted diet greatly increased the incidence of this phenomenon. Our results are thus consistent with previous observations of Seegmiller [8]. Age and gender of the experimental animals seemed not to affect susceptibility to s.i.b.

The need for a restricted diet, though, necessarily raises the possibility that a nutritional deficiency might be responsible to some extent for the production of s.i.b., particularly since one of 6 control rats on the reduced diet also showed this behaviour.

It is therefore especially significant that most (87%) of the Fischer rats tested showed s.i.b. as a result of caffeine administration, even though allowed free access to food and water. The onset of s.i.b. was also more rapid than in the semi-starved Wistars. Our second conclusion, therefore, is that Fischer 344 rats may be a much more suitable animal model of the Lesch-Nyhan syndrome, than semi-starved Wistars.

As to the cause of the s.i.b., an examination of Table 2 reveals few behaviours which bear any obvious relationship to the incidence of s.i.b. The phenomena which seem to occur mainly in those groups of animals showing s.i.b. include jumping, paw shake, jaw movements, teeth chattering, head movements, and tail erection. However, this behavioural profile of the rats showing s.i.b. does not suggest a primary involvement of any of the amines most studied for their behavioural effects, i.e. dopamine, noradrenaline or 5-hydroxytryptamine (5HT). For example, the constellation of behaviours usually associated with activity of 5HT neurones includes head weaving (or bobbing) and tremor, padding or kneading with the forepaws, abduction of the hindlimbs and Straub tail [3,10]. Of these, only head movements and Straub tail occurred with any regularity (Table 2) in the present tests. Also present, however, were repetitive jaw movements including licking and chewing, characteristic of dopamine receptor activation as also noted by Sakata and Fuchimoto during theophylline treatment [7]. It is therefore probable that methylxanthine administration enhances activity at both 5HT and dopamine receptors.

This suggestion is supported by the occurrence of backward walking, which has been shown by previous authors to be a likely consequence of the joint activation of 5HT and dopamine systems [2,4]. Furthermore amphetamine, well known for its ability to release all the biogenic amines, has very recently been found to produce self-injurious behaviour in rats, on chronic administration [6].

Finally the experiments of Waldeck [13] and of Berkowitz *et al.* [1] have yielded neurochemical evidence for an increased turnover of amines by methylxanthines. This action may in turn result from a blockade of presynaptic inhibitory receptors for adenosine [11,12]. We are currently attempting to interfere pharmacologically with the actions of the methylxanthines, in order to further clarify the involvement of amines in self-injurious behaviour.

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