INCREASED TOXICITY OF METHAM7PHETAMINE IN MORPHINE-DEPENDENT MICE

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Running title: Methamphetamine Toxicity in Morphinized Mice.

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Abstract

1. The effect of methamphetamine on morphine dependent mice was investigated by calculating the LD5O (i.p.), measuring motor activity, anorectic actions and body temperature.

2. Methamphetamine was more toxic in morphine-dependent mice *(LD5O* = 20.6 mg/kg) than in normal mice (LD5O = 43.2 mg/kg).

3. Methamphetamine induced locomotor activity was greater in morphinized than in non-morphinized mice at doses *of(2.5 & 5* mg/kg, i.p.).

4. Also methamphetamine increased the body temperature of morphinized mice to a higher degree than that of normal mice (P *<0.05).*

5. These findings suggest that methamphetaniine is more toxic in morphine-dependent as compared to non-dependent mice.

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LNThODUCTION

It is well known that the amphetamines enhance the effects of central monoanunes by a number of mechanisms, including blockade of their uptake, retardation of their breakdown and potentiation of their release (Glowinski, 1970). Many of the actions of these drugs are prevented or reduced by dopaminergic blocking agents, although the directly-acting dopaminergic agonists, such as apomorphine and bromocriptine, only partially substitute for amphetamine, in some of its actions.

Chronic morphine treatments have been shown to increase the sensitivity of central dopaniine receptors in mice (Martin and Takemori, 1986) and rats (Spampinato et al., 1988; Bhargava and Gulati, 1990). This supersensitivity has been observed as an increase in sensitivity to dopamine agonists (Eidelberg and Erspamer, 1975; Ritzmann et al., 1979, 1982a; Bhargava, 1980), and as increased affinity of striatal dopaniine receptors for the neuroleptic spiroperdol (Ritzmann et al., 1982b; Bhargava, 1983) in morphine-dependent rodents.

Morphine dependent subjects occasionally try to use another drug in combination with morphine or as a substitute for it. This is to avoid or suppress symptoms of morphine withdrawal or to gain its euphoric effect more rapidly. Use of one of the amphetamines or the neuroleptics is likely. However, the toxicity that might originate from such combinations remains unknowm The use of more than one drug among the opioid dependent addicts is a common practice that had often lead to catastrophic drug-drug interactions (Jasinski and Preston, 1986).

The aim of the present study is to investigate some of the toxic consequences of methamphetainine use in morphine-dependent mice. Predictably, methamphetaniine may produce different effects in morphine-dependent animals compared to appropriate controls, possibly due to the presence of dopamine supersensitive receptors in the former groups of animals.

MATERIALS AND **METHODS**

Male Swiss albino mice (obtained from the Animal Care Center, College of Pharmacy King Saud University) weighing 25-30 g were used. The animals were

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housed, 10 mice per cage, under conditions of constant room temperature (22±1 ^{0}C), humidity and light cycle (7a.m. to 7p.m.). They were given access to food (standard lab chow, Grain Silos and Flour Mills Organization, Riyadh) and water ad libitmn.

The following drugs were used morphine hydrochloride (Sandoz.S.A. Basle, Switzerland); naloxone hydrochloride (Fluka, Biochemika, Switzerland); 1 (+) methamphetaniine hydrochloride (E. Merck. Dermstadt, Germany); Drugs were dissolved in saline and administered i.p. in a volume of 10 mllkg

Mice were rendered morphine-dependent by twice daily i.p. injections for 3 days of morphine hydrochloride (40 mg/kg). Control animals received equal volumes of 0.9% NaC1 solution i.p in the same manner. Dependence to morphine was confirmed in some of the animals by the method of naloxone withdrawal jumping (Saelens ~t al, 1971) . Mice pretreated with morphine or saline were injected i.p. with 30 mg/kg of mialoxone hydrochloride, 2 hours after the last injection of morphine or saline. The animals were then individually placed in glass cylinders and the number of jumps by each animal subsequently recorded for a period of 10 minutes.

The median lethal dose *(LD5O)* for methamphetamine was determined in groups of mice (n=10 per group) made dependent to morphine but not challenged with naloxone. Methamphetamine was administered i.p., 24 hours after the last injection of morphine. Similarly, LD5O for methamphetainine was determined in groups of mice pretreated with saline (twice daily injections for 3 days)but not challenged with naloxone.

Mortality in animals was observed ever the next 24 hours. The LD5O was calculated using a basic computer program (Lieberman, 1983) dependent on

probit analysis technique.

Some of the animals at each dose level of methamphetamine were observed for changes in their behaviour using Irwin's procedure (1964). Also in some animals that survived the period of the LD5O test, the abdomen and chest were opened and examined for any gross pathological changes. Behavioural observation was from 30 mm. after methamphetainine injection until 1 h. later.

5 Locomotor activity was recorded using an activity meter (Optovarimex, Columbus Ohio, USA). The activity cage was equipped with horizontal and vertical sets of infrared photocells. The number of light beam intemiptions due to the animal's movements inside the cage were automatically recorded.

Groups of five mice each, either morphine dependent or saline treated, were given saline or various doses of methamphetaniine. Animals were then placed in the activity cage and counts of motor activity were recorded automatically every 10 minutes for two hours following saline or drug administration. Experiments were run between 10.00 a.m. and 3.00 p.m. (animals were awake and active during that period), under standard conditions of temperature, lighting and noise as was practicable.

Anorectic activity was measured in morphine dependent and control animals (treated with saline in a similar fashion) which were deprived of food for 24 hours. The animals, however, were allowed free access to water. Animals were divided randomly into groups of five. Each group was weighed and placed in a separate cage. Animals were injected with methamphetamine (or saline as appropriate). A weighed amount of the normal mouse food (z2Og) was placed at the top of the animals' cage. The amount of food intake per each group of mice was determined hourly for a period of *5* hours. This was done by reweigbing the food, at the top of the animal's cage, at 1 hour intervals. The difference between two successive readings indicated the food intake during that hour interval, hi order to standardize the measurements, food intake was determined as gm/3Ogm body weight of the mouse. Four groups of *5* animals

were used for each dose.

Rectal temperature was measured in morphine dependent and control animals treated with saline in a similar fashion. Animals were divided into groups of eight mice. Rectal temperature was measured at various times after drug treatment by gently inserting a lubricated temperature probe (model YSI 400) 3 cm into mouse rectum after allowing the probe to reach equilibrium (approximately 30 sec). The probe was attached to a digital thermometer (Apelex, Pb 0331, Panlab, France). Prior to any injection, baseline temperatures were taken 30 minutes before the drug or saline administration. The rectal temperatures were also measured at 30,

60 and 90 minutes post injection. All temperature measurements were made at room temperature (22 ± 1^0C). Each animal was used as its own control. Increases or decreases in body temperature were calculated by subtraction of the post-injection from the pre-injection rectal temperature readings.

Unless otherwise stated, statistical analysis was performed by using appropriate ANOVA tests (Winer, 1971). Post-hoc comparisons of any two means were made with the aid of the Student's t-test.

RESULTS

Development of dependence in mice:

The results of naloxone withdrawal jumping test in mice are presented in Table 1. From this table, the morphine-pretreated mice (40 mg/kg, i.p. twice daily for 3 days) showed a significantly higher number of responding animals than the saline-pretreated group• ($P<0.Ol$, using Chi-squared test, 2x2 contingency tables with Yate's correction), after being challenged with naloxone (30 mg/kg, i.p.). Also, the mean number of jumps elicited by naloxone in the morpbine-pretreated group was *22.5+4.6,* whereas no jumps were recorded in saline-pretreated mice. It is, therefore, clear that the morphine-pretreaUnent schedule used in this study was able to induce

dependence in mice, as evidenced by naloxone withdrawal jumping test. In all the subsequent experiments mice which received morphine pretreatment were assumed to be morphine-dependent (i.e. morphinized).

LD5O determination:

From Table 2, It is clear that methamphetaxnine was more toxic in morphine-dependent mice (LD5020.6 mg/kg) than in normal mice (LD5O=43 .2 mg/kg), when administered by the i.p. route.

No gross pathological changes were noted in morphinized and non-morphinized mice which were treated with methamphetamine(2.5 and *5* mg/kg, i.p.).

However a significant difference ($p<O.O5$) between the body weights of morphinized animals treated with methamphetaniine *(5* mg/kg, i.p) and non-morphinized mice treated with methamphetamine *(5* mg/kg, i.p.) was observed.

An increase in body weight of non-morphinized animals was observed whereas a decrease in body weight was noted in morphliuized animals (n=1 in each group).

Methamphetaxnine (2.5and5 mg/kg, i.p.) produced similar behavioural effects in morphinized and non-morphinized mice.These effects were characterized by increased alertness. However, stereotypic behaviour was only detected at the higher dose in both morphinized and non-morphimzed mice.Both doses of methamphetamine produced mood changes which were expressed as excessive grooming and by the presence of restlessness. Aggressive behaviour (demonstrated by fighting among the mice and the difficulty of handling them) was only increased at the higher dose *(5* mg/kg). In addition, animals showed hyperactivity and signs of increased CNS excitation. Methamphetaxnine (5mg/kg) produced greater responses to touch and pain, and more salivation in comparison to the dose of 2.5 mg/kg. Both doses were observed to cause piloerection and increased heart and respiratory rates in morphinized and non morphinized animals.

The effect of methamphetamine on the locomotor activity:

In animals which were not administered methamphetamine, the spontaneous locomotor activity of morphinized animals was significantly lower than that of non-morphinized animals ($P<0.05$) as shown in Fig. 1.

The effects of two doses of methamphetaniine (2.5 and *5* mg/kg, i.p.) administered to morphinized and non-morphinized mice are shown in figures 2 and 3, respectively. Methamphetamine markedly increased activity counts m both morphinized and non-morphinized animals. The effect of the lower dose of methamphetamine was significantly higher than the higher dose *($p<0.05$).* It was twice that of the higher dose. But no significant difference between methamphetamine-induced activity (both doses) in morphinized and non-morphinized animals was seen. Since the spontaneous activity of morphinized animals was significantly lower than that of non-morphimzed mice (Fig 1), it was concluded that methamphetamine-induced activity was greater in morphinized than in non-morphimzed mice.

The anor€ctic activity of methamphetamine

There were no significant differences in the food intake of control morphinized and non-morpbinized mice (Table 3.).

As expected, acute administeration of the anorectic agent methainphetamnine caused a decrease in the food intake of both morphinized and non-morphinized mice. From Table 3, it is clear that feeding ceased for the first hour after 2.5 mg/kg methamphetanune in morphinized and non-morphinized mice. By 2 hours the effect of methamphetainine was declining. Methamphetanune however, appeared to have a stronger anorectic activity in non-morphinized than in morphinized mice, since a significant difference was observed between the total food intake of morphinized and non-morphinized animals ($p<0.05$).

A higher dose of methamphetainine *(5* mg/kg) also caused significant

anorexia during the first hour after food presentation in both morpbinized and non-morphinized mice. Also, it seemed to have a stronger action in non-morphinized than in morphinized animals. No significant differences, however, between the effects of 2.5 and *5* mg/kg doses of methamphetamine in this test were calculated.

The effect of methamphetamine on body temperature:

In animals which were not administered methaniphetamine, there were no significant differences between the body temperatures of morphinized and non-morphinized mice.The results of three doses of methaxnphetamine (2.5, 5 and 10 mg/kg, i.p.) indicated that methamphetaniine was hyperthermic (pK).05.as compared to saline controls) in morphinized as well as non-morphinized mice, in a dose-dependent manner. Methaniphetamine, however, at doses of *5* and 10 mg/kg, i.p. did not produce significant differences between the body temperatures of morphinized and non-morphinized mice. Methamphetamine (2.5mg/kg), on ti_te other hand, significantly increased the body temperature of morphinized mice to a temperature higher than that recorded for the non-morphinized group ($p<O.O5$). The results of only the latter dose of methamphetamine are presented in Fig. 4.

DISCUSSION

The results of this study indicated that methamphetamine is more toxic -in morphine-dependent mice than in normal mice (LD5O i.p. values were 20.6 and 43.2 mg/kg, respectively). A significant difference between these LD5O values is indicated by their 95% confidence intervals (Table 2). It has previously been reported that the LD5O i.p. in mice for methamphetamine is 70 mg/kg (Lands et ai, 1947). The difference between the latter value and the results of this work may be attributed to use of different strains of mice. However the comparison in this study between animals of the same sex, strain and species with difference being only in a single factor, namely morphine-

dependence, is valid. Thus methaxnphetamine is predicted to have a lower margin of safety in morphinized than in non-morphinized animals.

No significant gi~oss behavioural differences between morphine-dependent mice (presumed to possess supersensitive dopamine receptors) and normal mice were observed. however, in some of the specific tests performed in this study clear differences were observed between the effects of rnethamphetamine *hi* morphine-dependent and non-dependent mice.

Administeration Qfmethamphetamine induced marked increases in locomotor activity of normal and morphine-dependent mice. From this study the effect ~f methamphetamine (2.5 and 5mg/kg) was significantly greater in morphine-dependent mice than in non-morphinized animals.

A dopaininergic action of the amphetamines rather than noradrenergic involvement in locomotor activity was claimed by many investigators (Randrup and Munkvad, 1967; Pijnenburg et at., 1975 a,b; Kelly et at., 1975).

The stimulant effect of methamphetamine in morphine-dependent mice conforms with the hypothesis of supersensitive dopamine receptors induced by repeated morphine treatment. Excessive CNS excitation is an undesirable effect of the amphetamines in many clinical situations, in particular when these are used in the suppression of appetite.

The amphetamines are believed to induce anorexia by their effects on dopaminergic systems in contrast to fenfluramine which acts via tryptaminergi

mechanisms to inhibit food intake (Garattini, 1980). The anorectic activity of methamphetamine was not modified in morphine-dependent animals, although these animals, suffered from a significant loss in their body weights.

In the present study, methamphetaniine induced a hyperthenmc effect in both saline controls and morphine-dependent mice. This effect of methamphetamine was dose-dependent. It was significantly higher in

morphine-dependent animals in comparison to normal mice at a low dose of methamphetamine (2.5 mg/kg). Relatively higher doses of methamphetaniine (5 and 10 mg/kg) did not show differences between dependent and normal mice.

Dopamine is claimed to play an important role in the central regulation of body temperature (Cox,1979; Clark and Lipton,1985; Lee et al.,1985). Considering that methamphetaniine is able via different mechanisms to increase central synaptic dopan~ine, it could be said that dopatnine is probably implicated in methamphetamine hyperthermia, as has previously been reported (Ageel and Ginawi, 1985).

Also, the increased hyperthermic effect of methamphetamine observed in morphine-dependent mice is probably due to increased central dopaminergic function either through more release of dopamine or via increased supersensitivity of postsynaptic dopamine receptors. Probably, excessive release of dopamine by higher doses of methamphetamine resulted in the disappearance of differences between the effects of methamphetamine in morphine-dependent and normal mice.

In conclusion the results of the present study indicated that the toxicity of methamphetainine was clearly greater in morphine-dependent animals because:

1) the LD5O i.p. was about one half that of normal animals,
2) the locomotor activity was more than that of normal animals (more restlessness and excitation), and
3) the body temperature was higher than that of normal animals.

Opioid addicts, therefore, should be warned of the serious consequences that may result in them after amphetamine use.

SUMMARY

A comparative study between the effects of acute methamphetamine hydrochloride in morphine-dependent and normal mice was performed. Mice were rendered dependent by twice daily intraperitoneal (i.p.) injections of morphine hydrochloride (40 mg/kg) for 3 days. Dependence was confirmed in

some animals by naloxone hydrochloride (30 mg/kg i.p.). Naloxone induced jumping only in morphine dependent mice. The median lethel dose (LD5O) of i.p. methamphetamine was significantly lower in morphine-dependent mice compared with that in normal mice (20.6 and 43.2 mg/kg. respectively). Also methamphetamine showed significantly greater effects on the locomotor activity and body temperature of morphine-dependent animals. Food intake of fasted mice, which were treated with methamphetamine 24 hours after occurrence of dependence to morphine, was not modified by morphine-dependence. Methamphetamine, therefore, appeared to have a greater toxicity in morphinized animals than in normal mice. Opioid addicts need a warning about methamphetamine use because of the expected serious consequences.

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