EFFECT OF APAMIN ON TOLERANCE TO COCAINE-INDUCED LOCOMOTOR ACTIVITY IN MICE

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Abstract- In the present study, the effect of apamin (potassium channel blocker) on tolerance to cocaine-induced locomotor activity in mice has been investigated. Locomotor activity was measured by locomotor activity meter, Animax, type S (LKB, Farrad). Intraperitoneal (IP) injection of different doses of cocaine (2.5, 5, 10 and 15 mg/kg) produced dose-dependent locomotor activity in mice. Animals were treated with a dose of cocaine (60 mg/kg, IP) once daily, for 2, 3 or 4 days in order to produce tolerance to cocaine-induced locomotion. Twenty-four hours after the last dose of cocaine, locomotor activity induced by a test dose of cocaine (10 mg/kg) was assessed. Animals pretreated with apamin (0.1 mg/kg) 30 min before the test dose of cocaine had a decreased cocaine response. However, daily treatment of animals with apamin (0.1 mg/kg), 30 min after cocaine (60 mg/kg) for 3 days (during development of tolerance to cocaine-induced locomotion), did not alter the cocaine effect. Single administration of apamin to mice did not cause any response. It is concluded that, apamin as a potassium channels blocker may decrease tolerance to cocaine-induced locomotion due to blockade of potassium channels.

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Key words: Cocaine, apamin, tolerance, locomotor activity, mice

INTRODUCTION

Cocaine has several sites of action in the central nervous system. It is a widely abused drug, presumably because of its euphoriant and stimulant properties (1,2). The drug causes reinforcing properties (3-5) through the mesolimbic-dopaminergic system (2,6,7). It exerts behavioral effects, at least in part, by binding to the dopamine transporter, blocking synaptic dopamine reuptake, and thereby potentiating dopaminergic neurotransmission (6). It is well established that cocaine increases locomotor activity and produces stereotyped behavior in animals (8). However, repeated administration of low to moderate doses of cocaine enhances these responses (9-14) Tolerance to locomotor effects of cocaine has also been reported to develop when the drug is administered by continuous infusion (15,16). The dose, route, and frequency of cocaine administration, environmental context and behavior being measured, may be important factors in inducing tolerance or sensitization to cocaine response (17). There is a report indicating that various K^+ channels contribute to the generation of locomotor activity (18). There are a variety of different subtypes of K^+ channels, which have been classified on the basis of their voltage-gating properties, second messenger regulation, and sensitivity to calcium. Blockade of K^+ channels can prolong the duration of action potentials, which leads to delayed closure of calcium channels, increases calcium influx and thus increases the release of transmitter (18).

It has been suggested the various K^+ channels contribute differentially to the generation of locomotor activity (19). Apamin, a neurotoxic polypeptide isolated from bee venom (20) has been mainly shown to block K^+ channels (21). The action

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of apamin appears to block only the small conductance Ca^{2+}-activated K^+ channels responsible for slow after- hyperpolarization (19). In the present study, the influence of apamin on cocaine-induced tolerance has been investigated.

MATERIALS AND METHODS

Animals: Male albino mice (20-30 g) were used in these experiments. There were nine mice in each group. They were kept 10 per cage (45 x 30 x 15 cm) at an environmental temperature of 22-24 °C on a 12 hour light-dark cycle.

The animals had free access to food and water, except during the experiments. Each animal was used once only and was euthanized immediately after the experiments.

Development of tolerance by cocaine

Tolerance to cocaine-induced locomotor activity was achieved by daily administration of cocaine (60 mg/kg) for 3 days. To assess the tolerance, locomotor activity induced by a test dose of cocaine (10 mg/kg, IP) was measured on the 4th day after injection of the drug (16).

Locomotor activity measurement

Locomotion was measured with an activity meter, Animex, Type S (LKB Farad). Each animal was placed in a plastic cage for 15 min to acclimatize to the environment. Immediately after drug injection, the animals were returned to the cage for test trial lasting 60 min.

Drugs

Cocaine hydrochloride (May & Baker, England) was used to induce tolerance to the drug. The drugs were given intraperitoneally, in a volume of 10 ml/kg and were prepared immediately before use.

Apamin was prepared from Physiology Institute of Active Compounds in Russia.

Analysis of data

Comparison between groups was made with Newman-Keuls test, following ANOVAs. Differences with $p<0.05$ between experimental groups at each point were considered statistically significant.

RESULTS

Locomotor activity induced by cocaine in intact tolerant mice

Figure 1 illustrates the locomotion induced by intraperitoneal (IP) injection of different doses of cocaine (2.5, 5, 10 and 15 mg/kg). One-way ANOVA showed that cocaine induced a significant increase in locomotion [$F(4,40)=8.1$, $p<0.0001$].

Table 1 indicates the response of test doses of cocaine in tolerant animals. Mice were injected IP with cocaine hydrochloride (60 mg/kg) once daily, for 2, 3 or 4 days, in order to produce tolerance to cocaine-induced locomotion. The test dose of cocaine (10 mg/kg, IP) was tested on the 3rd, 4th, or 5th day, 24 h after the last dose of cocaine. Animals which had become tolerant to cocaine showed only small locomotor activity in response to the test dose of cocaine. [One-Way ANOVA; $F(5, 48)=21.3$, $p<0.0001$].

Effect of apamin on chronic cocaine-treated animals

Figure 2 shows the effect of apamin on expression of tolerance to cocaine. In order to induce tolerance, the mice were injected daily with cocaine (60 mg/kg, IP), for a period of 3 days. Locomotor activity response of the test dose of cocaine (10 mg/kg, IP) was tested on the 4th day.

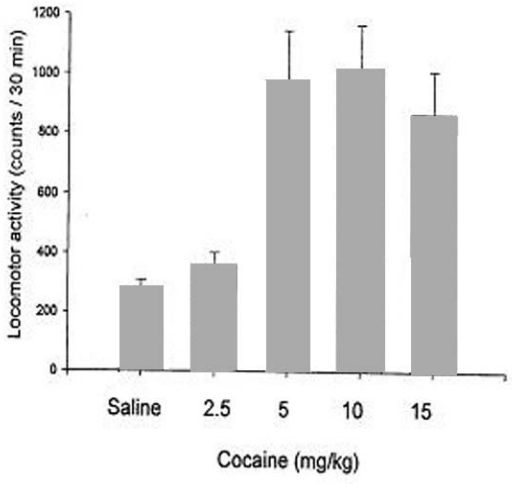

Fig. 1. Dose-response effect of cocaine

Table 1. Tolerance induced by cocaine during 2, 3, 4 days

	Saline	Cocaine 2.5 mg/kg	Cocaine 5 mg/kg	Cocaine 10 mg/kg	Cocaine 15 mg/kg
Mean :	281.11	358.66	978.22	1017.33	865.55
SE:	26.70	43.44	167.16	149.22	145.57
		saline			
	2 days saline	3 days saline	4 days saline		
Mean :	2130.42	1645.42	1773.42		
SE:	171.86	174.63	157.91		
		Cocaine 60 (mg/kg)			
	2 days cocaine	3 days cocaine	4 days cocaine		
Mean :	794.66	608.57	765.77		
SE:	140.42	92.9	48.541		
	Saline (cocaine 60 mg/kg)	Apamin+ Cocaine (DEV)	Apamin+ Cocaine (EXP)		
Mean :	608.57	696.14	1318.87		
SE:	92.9	118.49	107.52		

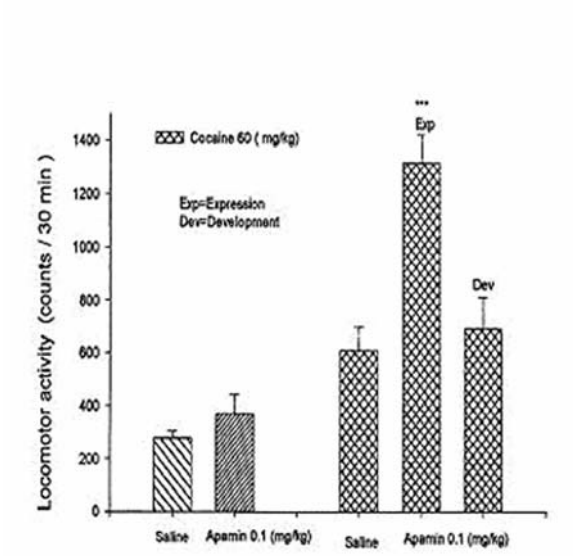

Fig. 2. Effect of apamin on tolerance induced by cocaine

One-way ANOVA showed a significant difference between the response to cocaine in the presence or absence of apamin, regarding the expression of tolerance to cocaine-induced increase in locomotion [$F(4, 35)=22.1$, $p<0.0001$]. Further analysis indicated that administration of apamin (0.1 mg/kg) before the test dose of cocaine (10 mg/kg) decreased tolerance to cocaine, while apamin administration during development did not alter the tolerance to the drug.

DISCUSSION

It has been shown that cocaine increases locomotor and stereotypic activity in animals (8). The development of either tolerance (22,23) or sensitization (17,24) to cocaine's behavioral effects has been reported. Locomotor activity induced by cocaine may be mediated through dopaminergic system (2,7,25,26). Moreover, high doses of apamin have been shown to increase extracellular dopamine concentration (18). In the present study, the effect of apamin, a potassium channel blocker, on tolerance to cocaine-induced locomotion has been studied. Our data showed that different doses of cocaine increased locomotion in mice. The results are in agreement with others (27) in this respect. Several reports suggested that behavioral

effect of cocaine might result from alterations in mesolimbic and nigrostriatal dopamine and their response to subsequent cocaine administration. Cocaine exerts its potent reinforcing properties through the mesolimbic-dopaminergic system, by binding to the dopamine transporter and blocking the neuronal uptake of dopamine (2,6,7,25). Behavioral sensitization to cocaine has been related to enhanced extracellular dopamine levels following a cocaine challenge (9,11,28-30). In contrast, continuous infusion of cocaine results in decreased extracellular levels of dopamine during perfusion of striatal slices of cocaine (30). The mechanism underlying different behavioral and neurochemical effects of cocaine is not known.

Dopaminergic mechanisms may be essential for drugs to elicit locomotor activity (26). The locomotor activity induced by cocaine may therefore be induced through dopaminergic mechanisms. Although repeated doses of cocaine have been shown to cause a reverse tolerance or enhanced sensitivity to the locomotor effects of a subsequent dose of cocaine (8), our study showed that repeated administration with higher doses of cocaine reduced locomotor activity induced by the test dose of the drug, indicating tolerance to its effect. This is in agreement with the authors who showed that tolerance to cocaine might be elicited by up-regulation of dopamine receptors (22,23).

Similar to cocaine, subchronic treatment of apomorphine (0.5 mg/kg), which is D1/D2 dopamine receptor agonist, decreased the effect of the test dose of apomorphine (unpublished data). In fact, this may indicate that chronic treatment of dopaminergic agonists produces a down-regulation of dopaminergic receptors. Further testing is required to determine whether the tolerance to cocaine-induced locomotor activity is produced through similar mechanisms.

This hypothesis is supported by evidence suggesting that continuous cocaine administration produces tolerance to the subsequent behavioral and neurochemical (e.g., blockade of dopamine reuptake) effects associated with D2 autoreceptors (22).

The dose, route of administration, and frequency of cocaine administration, as well as the environmental context and behavior being measured, have been proposed to be important in the development of tolerance or sensitization (17). Recent studies have demonstrated that the development of behavioral sensitization may be dependent on changes that occur in the ventral tegmental area (VTA). Apamin, a neurotoxic polypeptide isolated from bee venom has been mainly shown to block K^+channels (21). The effect of apamin and other K^+ channel blockers during sequences of locomotor-like activity has been investigated and it has been concluded that various K^+ channels contribute to the generation of locomotor activity (18).

In the present study, the effect of apamin on cocaine and the expression and development of tolerance to cocaine was studied. The data indicated that apamin could inhibit the expression, but not the development of tolerance to cocaine. Administering different doses of apamin (0.05, and 0.1 mg/kg) did not have any effect on locomotor activity.

During development of tolerance to cocaine (long-term administration of 60 mg/kg), apamin did not have any effect on tolerance. Therefore, it can be postulated that potassium channels are not involved in the development of tolerance. However, when apamin was employed before expression of cocaine, the response of the drug was increased indicating that apamin might only increase the drug response.

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