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The Clonidine-Induced Self-Injurious Behavior of Mice Involves Purinergic Mechanisms

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KATSURAGI, T , I. USHIJIMA AND T FURUKAWA *The clonidine-induced self-injurious behavior of mice involves purinergic mechanisms*. PHARMACOL BIOCHEM BEHAV **20**(6)943–946, 1984.— Mode of action of clonidine involved in self-injurious behavior was assessed in mice. Single injection of clonidine (50 mg/kg, IP) evoked a self-biting which occurred more frequently under the condition of isolation and food-deprivation for 24 hr. The clonidine-induced self-biting was not reduced, but rather potentiated by pretreatment with phentolamine (10 mg/kg). This behavior was enhanced by theophylline (20 mg/kg) but was inhibited to some extent by adenosine (10 mg/kg) or dipyridamole (10 mg/kg). In addition, the self-injurious behavior was completely antagonized by combined pretreatment with adenosine (10 mg/kg) and dipyridamole (10 mg/kg), and by potent adenosine agonists, such as N^6-(L-phenylisopropyl) adenosine (0.2 mg/kg) and N^6-cyclohexyladenosine (0.2 mg/kg). These results, therefore, suggest that the clonidine-induced self-biting could be substantially attributed to adenosine A_1-receptor blockade as documented for pharmacological property of theophylline in the brain

Clonidine Theophylline Adenosine Cyclohexyladenosine L-Phenylisopropyladenosine
Self-biting Mouse

SELF-INJURIOUS behaviors including self-biting in non-human mammals have been investigated to define the principal biological and social correlates and then to consider what can be inferred from animal studies about deliberate self-injury in man [9]. Artificial environments, such as laboratory and zoo [16], predispose animals to self-injurious behavior. The laboratory-reared male monkeys showed self-biting in 50% of the observation sessions, females in 35%, but non-reared animals showed virtually no self-damage [22]. In macaques, the more severe injury is likely to be accompanied by other aggressive acts and the behavior has mainly been reported in situations of confinement or isolation [1]. Self-mutilation in human occurs in about 10% of the severely retarded population [5], but occurs at a much higher frequency in the patients who exhibit mental and physical retardation with the de Lange syndrome [17], Lesch-Nyhan syndrome [13] of childhood and schizophrenics [9].

Such behavior as self-biting or self-mutilation has also been reported to be elicited by treatment with drugs in animals. The behavior was produced by alkylxanthines, such as caffeine and theophylline, in rats [3,14], and by pemoline in rats and mice [17,18]. Clonidine, an anti-hypertensive drug, exerts interesting multi-actions at synaps, including stimulation of adrenergic α-receptor [11] and inhibition of uptake$_2$ process of catecholamines [23]. This drug also allows mice to injure themselves in the absence of objects to bite. Following intraperitoneal administration of 50 mg/kg clonidine, mice bite off their digits, forelimbs and sometimes hindlimbs, and this biting behavior might be, in part, due to facilitation of central noradrenergic mechanism and inhibition of cholinergic mechanism [20,21]. However, clonidine like theophylline, presynaptically facilitates ^{3}H-purine release from the rabbit vascular adrenergic nerves, presumably by blocking P_1-purinoceptor [10]. From the microiontophoretical approach, clonidine seems to be block adenosine receptor in the rat brain [27,28]. Accordingly neurological mechanisms related to the clonidine-induced self-biting still remains to be determined.

The present study was designed to understand central purinergic mechanisms involved in self-biting induced by a high dose of clonidine in mice.

METHOD

Animals

Healthy male ddY albino mice (30–35 g) purchased from Kyudo Animal Laboratory (Kumamoto, Japan) were permitted food (CE-2, Clea, Ltd., Japan) and tap water ad lib except during trials. All trials and breeding were carried out at an environmental temperature of 24±1°C, with a 12 hr light-dark cycle. After mice had been caged in a group of five, they were individually housed in each plastic cage where food was deprived for 24 hr before the experiments, except for the control mouse.

Evaluation of Self-Mutilation

Mice received drug were placed in a transparent plastic box (21.5×32×13 cm) for 10 min. Then, aggressive mice

TABLE 1

SELF-BITING INDUCED BY CLONIDINE ALONE OR IN COMBINATION WITH THEOPHYLLINE IN MICE IN THE NORMAL OR ISOLATED AND FOOD-DEPRIVED CONDITION

	% Incidence			
	A		B	
Drugs (mg/kg)	Saline	Theophylline (20)	Saline	Theophylline (20)
Saline	0	0	0	0
Clonidine (30)	—	—	0	10
Clonidine (40)	—	—	20	30
Clonidine (50)	30	50*	50*	70*

A: mice were used in the condition without any restriction

B mice were used after isolation and food-deprivation for 24 hr before the experiments

Each value means % incidence of self-biting observed in ten mice during 30 min after clonidine

*$p<0.05$, significant difference from the corresponding saline-injected group, calculating by the Fisher exact probability test

were picked up, placed on a smooth surface table and covered individually with a transparent grass beaker of 15 cm in diameter for behavioral observation as reported by Razzak *et al* [20]. Self-biting behavior in mouse was evaluated as incidence of responses, such as nibbling and biting the frontal body, especially spreaded digits of the limbs, for 30 min after clonidine.

Administration of Drugs

Throughout the experiment, all test drugs including clonidine were intraperitoneally injected to mouse in the volume of up to 0.2 ml. In the experiment of clonidine plus theophylline, theophrine was administered 10 min before clonidine. To evaluate effects of various drugs on the clonidine-induced self-biting, phentolamine or dipyridamole and N^6-cyclohexyladenosine, N^6-(L-phenylisopropyl) adenosine or adenosine were injected 30 and 15 min prior to clonidine, respectively. These N^6-substituted adenosine analogs because of insolubility were used as suspension with 0.3% carboxymethylcellulose. The solvent did not appreciably affect normal behavior of mouse.

Drugs

Clonidine hydrochloride (gift from Boehringer-Ingelheim), theophylline (Nakarai), phentolamine mesylate (Regitine; Ciba-Geigy), N^6-cyclohexyladenosine (CHA; Calbiochem), N^6-(L-phenylisopropyl) adenosine (L-PIA; Boehringer-Mannheim), adenosine (Sigma) and dipyridamole (Boehringer-Ingelheim) were used.

Statistical Analysis

Data were analyzed by means of 2×2 contingency table and Fisher exact probability test and the level of significance chosen was $p<0.05$.

RESULTS

Self-Biting After Clonidine or Clonidine Plus Theophylline

Mice manifested aggressive behavioral responses such as biting and fighting each other about 5 min after single administration of clonidine (50 mg/kg, IP). Subsequently, when the aggressive mice were individually separated 10 min after clonidine, self-biting occurred. The mice nibbled, bit and cut off their own digits of the forelimbs and occasionally shrieked. The self-biting was preceded by abnormal grooming, preening, and nibbling table or grass beaker. This biting behavior usually began within 10–15 min and reached to a higher frequency 20–30 min after the drugs.

The self-biting produced by clonidine (50 mg/kg) was increased by procedure of isolation and food-deprivation for 24 hr in preference to the experiment. Dose-response relations between clonidine administered alone or in combination with theophylline and occurrence of self-biting behavior were assessed in isolated and food deprived mice. The clonidine-induced self-biting was increased in a dose dependent manner though in narrow dose range from 30 to 50 mg/kg. The response at each dose was enhanced by preinjection of theophylline (20 mg/kg) 10 min before clonidine (Table 1).

Effects of Various Drugs on the Self-Biting Induced by Clonidine or Clonidine Plus Theophylline

The results are summarized in Table 2. In the isolated and food-deprived condition, the self-biting elicited by clonidine (50 mg/kg) alone or in combination with theophylline (20 mg/kg) was increased to a certain degree by pretreatment with phentolamine (10 mg/kg) but this increase was not significant. The self-biting was markedly inhibited by CHA (0.1 and 0.2 mg/kg) or L-PIA (0.2 mg/kg) at the doses without producing a sedation and muscle relaxation. Adenosine (10 mg/kg) or dipyridamole (2 mg/kg) inhibited slightly, but not significantly the self-biting. However, when adenosine was administered in combination with dipyridamole, the self-biting was completely inhibited. In addition, aggressive responses such as biting and fighting, grooming and preening were similarly suppressed by these drug treatments.

DISCUSSION

Clonidine, a unique antihypertensive drug, has been proposed to stimulate α-adrenoceptors in the central nervous system [8,31]. Lower doses of clonidine preferentially activate presynaptic α_2-adrenoceptors which result in an inhibition of noradrenaline release [11], while higher doses stimulate postsynaptic α_1-adrenoceptors [2, 12, 29]. Parallel

TABLE 2

EFFECTS OF DRUGS ON SELF-BITING INDUCED BY CLONIDINE ALONE OR IN COMBINATION WITH THEOPHYLLINE IN MICE

Drugs (mg/kg)	% Incidence: Clonidine (50)	% Incidence: Theophylline (20) + Clonidine (50)
Saline	50	70
Phentolamine (10)	70	90
CHA (0.1)	0*	10*
CHA (0 2)	0*	0*
L-PIA (0.2)	0*	0*
Dipyridamole (10)	30	40
Adenosine (10)	40	40
Adenosine (10) + Dipyridamole (10)	0*	0*

Test drugs were injected as described in text under the condition of isolation and food-deprivation.

Further explanation as in Table 1

to these effects, clonidine produces a marked sedation at low doses in human [24], but an aggressive behavior, hypermotility, sizable tremor and piloerection at higher doses in mice [15].

In the present study, after a large dose of clonidine (50 mg/kg), mice which were placed in a pair exhibited aggressive behavior such as biting and fighting each other. When mouse was individually placed on a smooth surface table in the absence of objects to bite, clonidine produced self-biting as reported by Ueki and his colleagues [20,21]. The frequency of self-biting was increased under the condition of isolation and food-deprivation for 24 hr.

The self-biting induced by clonidine in the presence or absence of theophylline was not reduced and rather enhanced by phentolamine, a nonspecific α_1 and α_2-adrenoceptors blocking agent [26], implying minimized relationship between the occurrence of self-biting behavior and stimulation of α-adrenoceptors, especially α_2-receptor, by clonidine although α_2-adrenoceptor antagonist such as yohimbine was not tried. The self-biting was potentiated by pretreatment with theophylline (20 mg/kg). These results suggest that clonidine may exert an action through the similar mechanism to these of theophylline, an adenosine antagonist.

In the past five years, Stone and Taylor [27,28] have reported that clonidine may be an antiadenosine agent because an inhibition by adenosine of the spike discharge from the rat cortical neuron is counteracted by clonidine. Supportive data were later presented by Katsuragi and Su [10] that clonidine as well as theophylline presynaptically facilitates ^{3}H-purine release from vascular adrenergic nerves, presumably via blockade of P_1-purinoceptor postulated by Burnstock [6]. Furthermore, adenosine seems to play an important role in regulating the central nervous functions, since the substance displays antilocomotive, analgesic and sedative effects which are antagonized by theophylline [25,32]. On the contrary, theophylline elicits convulsion in mammalians [7].

In the present experiments, dipyridamole, a blocker of adenosine uptake, counteracted, to some extent, the clonidine-induced self-biting, indicating possible contribution of endogenous adenosine to improve this abnormal behavior. Exogenously administered adenosine also exerted, to a certain degree, the inhibitory action. This mild counteractive effect of adenosine seems to be largely due to rapid uptake of the nucleoside into the cells. In fact, when adenosine administered in combination with dipyridamole, the self-biting behavior was completely eliminated. In addition, N^6-substituted analogs of adenosine, CHA and L-PIA, which are highly resistant to inactivation by adenosine deaminase [32] and unable to be taken up into the cells of nervous tissues [4], almost abolished the self-biting induced by clonidine. The grooming and preening induced by clonidine or clonidine plus theophylline were also reduced by these drugs. From these findings, the self-biting elicited by clonidine appears to involve obvious antiadenosine action. In recent years, purinergic receptors have been classified into two different types and termed as A_1 and A_2-receptors on the basis of inhibitory and stimulatory effects of adenosine on rat brain adenylate cyclase activity, respectively [29]. CHA and L-PIA are potent agonists for A_1-receptor which corresponds to P_1-receptor in the peripheral nerves [19], whereas alkylxanthines, caffeine and theophylline, are antagonists for both A_1 and A_2-adenosine receptors [7].

The present results strengthen the concept that higher doses of clonidine, like alkylxanthines, elicits an antiadenosine action in the central nervous system and thereby produces a self-biting behavior by inhibiting presynaptic purine receptors, probably A_1-receptor.

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