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Selective Antagonism by Clonidine of the Stereotyped and Non-Stereotyped Motor Activity Elicited by Atropine

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MOLLOY, A. G., R. S. ARONSTAM AND J. J. BUCCAFUSCO. *Selective antagonism by clonidine of the stereotyped and non-stereotyped motor activity elicited by atropine.* PHARMACOL BIOCHEM BEHAV **25**(5) 985–988, 1986.—The effects of clonidine, an indirectly-acting cholinergic antagonist, on 5 behaviors elicited by atropine (locomotion, rearing, sniffing, grooming and gnawing) were studied in rats. Clonidine did not alter the prevalence or magnitude of atropine-elicited locomotion and rearing. In contrast, clonidine suppressed the occurrence and degree of 3 stereotyped behaviors, namely, sniffing, grooming and gnawing. This selectivity of clonidine suggests differences in the neural pathways subserving the various stereotyped motor activities.

Clonidine Atropine Stereotypy Behavior Locomotor activity

CLONIDINE is widely employed as an antihypertensive drug which lowers arterial blood pressure by an action on alpha-adrenergic receptors within the central nervous system. This beneficial action is often associated with side effects which resemble those produced by anticholinergic drugs, including dry mouth, sedation and urinary retention [16]. Studies in this laboratory have demonstrated an interaction of clonidine and related drugs with central cholinergic neurons. This presynaptic interaction is mediated by central alpha-adrenergic receptors and results in a reduction in the synthesis and release of acetylcholine in certain brain regions [2–4]. The inhibitory action of clonidine on central cholinergic neurons is pharmacologically significant since pretreatment with this drug reduces the toxicity of centrally acting acetylcholinesterase inhibitors, such as physostigmine [3]. In fact, clonidine can potentiate the protective actions of atropine, a classical antidote to cholinesterase inhibitor poisoning [5].

While studying the use of clonidine and atropine in cholinesterase poisoning, the animals exhibited reduced atropine side effects when clonidine was administered just prior to the muscarinic antagonist. In another previous study [7] atropine at a dose of 5–10 mg/kg was found to antagonize the sedative action of 0.75 mg/kg of clonidine in mice. These initial observations suggest an inhibitory effect of clonidine pretreatment on the motor behaviors promoted by atropine. Therefore, the present study was designed to quantitate the effect of clonidine on the individual motor behaviors promoted by atropine. Our method of observation and analysis was capable of describing both the nature of the composite behavioral response to atropine, and of quantifying the individual behaviors exhibited in an experimental group (prevalences) and within individual animals (counts).

METHOD

Male, outbred Wistar rats were obtained from Harlan Sprague-Dawley and housed in our animal facilities for at least one week prior to the experiment. The animals were maintained on a 12 hr:12 hr light:dark cycle and had access to an unlimited supply of tap water and standard chow (Wayne Rodent Blox). At 9–12 weeks of age rats were habituated in individual observation cages for 2.5 hr just prior to the experiment.

Assessment Procedure

At specified intervals after challenge with vehicle or drug (see below), animals were assessed visually employing a rapid time sampling behavioral check list and a stereotypy rating scale described in detail elsewhere [11, 13, 15]. Briefly, each rat was observed for 10 sec periods at 1 min intervals over 5 consecutive minutes using the checklist. The presence or absence of sniffing, locomotion, rearing, grooming and gnawing (occurring alone or in combination) was

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TABLE 1

PREVALENCE OF INDIVIDUAL BEHAVIORS PROMOTED BY ATROPINE

Treatment (mg/kg)	Prevalence of Behaviors				
	Sniffing	Locomotion	Rearing	Grooming	Gnawing
Vehicle	1/6	0/6	0/6	1/6	0/6
Atropine (6)	6/6†	5/6†	5/6†	5/6*	4/6*
Clonidine (0.2)	1/6	2/6	3/6	0/6	0/6
Clonidine + Atropine	6/6†	5/6†	5/6†	0/6§	0/6§
Atropine (25)	5/5*	5/5†	5/5†	3/5	4/5*
Clonidine + Atropine	4/6	4/6*	4/6*	0/6	0/6‡

Prevalences for sniffing, locomotion and rearing were obtained at 30 min after injection. Prevalence for grooming and gnawing were obtained at, respectively, 24 and 18 min after atropine injection. Clonidine was injected 5 min prior to atropine.

*$p<0.05$, †$p<0.01$ as compared to vehicle control values.

‡$p<0.05$, §$p<0.01$ as compared to atropine alone value.

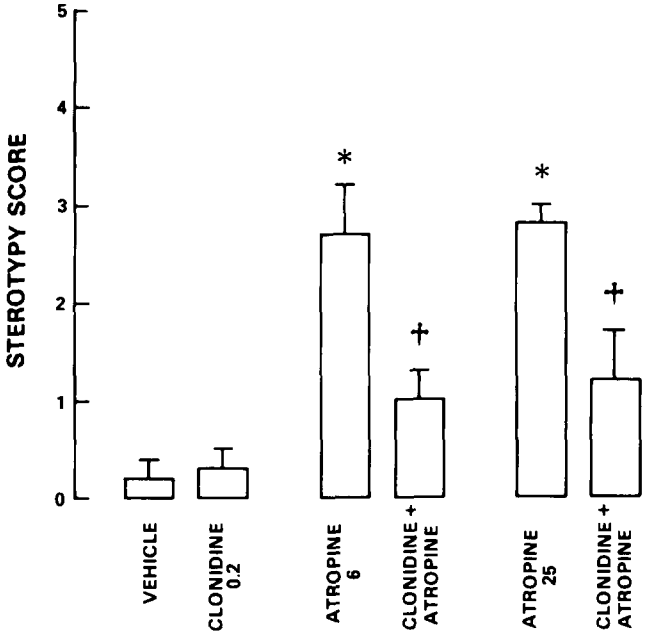

FIG. 1. Stereotypy scores (mean±S.E.M.) reflecting the peak stereotypy response exhibited by animals (n=5–6) injected with vehicle, atropine (at 6 or 25 mg/kg) or atropine plus clonidine (0.2 mg/kg). Vehicle or clonidine was administered 5 min before atropine. *$p<0.01$, compared to vehicle or clonidine alone treatments; †$p<0.05$ compared to respective atropine treatment.

determined. The grooming behavior had a characteristic pattern of wiping the head or face with the forepaws and/or scratching the flank with the hind paw. After the assessment of the individual behaviors using the checklist, the composite behavior of the animals was analyzed using the following 0–6 point stereotypy rating scale: 0=asleep or inactive; 1=episodes of normal activities; 2=discontinuous activity with bursts of prominent sniffing, grooming and rearings; 3=continuous stereotyped activity such as sniffing or rearing along a fixed path; 4=stereotyped sniffing or rearing in a fixed location; 5=stereotyped behavior with bursts of licking and gnawing; 6=continuous licking or gnawing.

Experimental Protocol

Atropine sulfate (6 or 25 mg/kg) or vehicle (normal saline, 2 ml/kg) was injected subcutaneously into the flank 30 min prior to the onset of behavioral observations. This dose range for atropine was chosen because it is approximately equivalent to the dose of clonidine hydrochloride (0.2 mg/kg) given to protect against cholinesterase inhibitor toxicity [5], and because it is similar to that which reversed the sedative effect of clonidine in mice [7]. Clonidine or vehicle (saline) was always administered SC 5 min prior to atropine.

Statistical Analysis

The rapid sampling checklist procedure provided two basic parameters: (1) the presence of a given individual behavior occurring on at least one occasion within the 5 consecutive observations and expressed as the ratio of the number of animals exhibiting the behavior to the total group size (n=5–6), repeated for each 6 min interval; (2) the number of individual behaviors determined as the number of observations in which a given behavior occurred (up to a maximum of 5 for each time point) and summed over the appropriate time points. Parametric data are expressed as the mean±S.E.M. and means were compared using the Mann-Whitney U test. Frequency data were compared using a Four-Fold Table. In both cases, data between groups were considered significantly different at the $p<0.05$ level. Data is compared only from time points or time intervals during which the maximal behavioral changes were observed following atropine administration (generally 18–30 min).

RESULTS

Preconditioning resulted in low basal stereotypy scores for control animals (Fig. 1). Animals injected with either dose of atropine, however, exhibited several behaviors described as stereotyped, i.e., repetitive, invariant and inappropriate (mean scores greater than 2.0). The peak stereotypy responses occurred at 12 min after atropine administration. Clonidine did not evoke significant stereotyped

TABLE 2

INTENSITY OF INDIVIDUAL BEHAVIORS PROMOTED BY ATROPINE

Treatment (mg/kg)	Behavioral Counts (mean ± S.E.M.)				
	Sniffing	Locomotion	Rearing	Grooming	Gnawing
Vehicle	2.3 ± 1.7	0.8 ± 0.8	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
Atropine (6)	13.8 ± 1.6†	5.2 ± 2.9*	9.0 ± 1.5†	2.8 ± 1.5†	2.8 ± 1.1†
Clonidine (0.2)	1.0 ± 0.2	1.2 ± 0.4	1.7 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
Clonidine + Atropine	7.7 ± 2.1‡	4.8 ± 1.8*	6.3 ± 1.5*	0.0 ± 0.0‡	0.0 ± 0.0§
Atropine (25)	12.6 ± 0.7†	7.8 ± 1.8†	6.2 ± 1.5†	4.6 ± 1.6†	4.0 ± 0.4†
Clonidine + Atropine	7.0 ± 1.7†	5.8 ± 1.5*	4.8 ± 1.6*	0.2 ± 0.2‡	0.0 ± 0.0§

Behavioral counts between 18 and 30 min post injection are presented.
*$p<0.05$, †$p<0.01$ as compared to vehicle control values.
‡$p<0.05$, §$p<0.01$ as compared to atropine alone value.

behavior. Pretreatment with clonidine, however, significantly reduced the behavior provoked by atropine. In fact, the stereotypy score for the combination of atropine and clonidine was not significantly greater than that for saline control animals.

Table 1 lists the data for the prevalence (at their respective maximal time of occurrence) for five behaviors: sniffing, locomotion, rearing, grooming and gnawing. Significant increases (compared with saline control animals) in sniffing, locomotion and rearing were observed following atropine administration, with these behaviors being promoted maximally at 30 min after injection. Clonidine pretreatment, however, did not reverse the occurrence of these behaviors in the population. Atropine also induced signs of grooming and gnawing in most of the animals examined. These behaviors were promoted maximally at 24 and 18 min post injection, respectively. Clonidine effectively prevented atropine elicitation of these two behaviors (Table 1).

The number of times each behavior was observed between 18 and 30 min (time period of maximal frequency of composite behavior) of observation were expressed as behavioral counts (Table 2). Atropine treated rats exhibited significantly increased frequencies of all 5 behaviors compared with control (vehicle-treated) rats. As with the prevalence data, clonidine pretreatment significantly reduced the frequency of grooming and gnawing, without affecting the frequency of locomotion or rearing. Clonidine pretreatment reduced but did not abolish the frequency of sniffing in response to atropine.

DISCUSSION

The stereotyped nature of atropine induced behavioral changes has been described previously [9,17]. These actions occur at relatively high dose levels (5–50 mg/kg) and are potentiated in animals in which cholinergic receptor down regulation has been induced by chronic administration of cholinergic agonists [9]. The profile of behavioral changes associated with atropine induced stereotypy is unlike, in several respects, those associated with dopaminergic or serotonergic stereotypy ([14] and see [9]). In addition, clonidine is not acting in a manner similar to amphetamine, since clonidine does not induce stereotyped behaviors, and since amphetamine administration enhances, rather than inhibits, stereotyped behavior promoted by atropine [1].

The present study is the first to demonstrate an inhibitory action of clonidine on atropine-induced stereotypy. Clonidine is selective in its effects on atropine-elicited behaviors: Sniffing, gnawing and grooming are depressed, while locomotion and rearing are unaffected. It is clear from our analysis of the behavioral checklist that whereas clonidine pretreatment does not alter the prevalence of sniffing promoted by atropine, clonidine does antagonize the extent to which individual animals emit this behavior. The similar effect of clonidine pretreatment in reducing atropine induced grooming and gnawing suggests that these three behaviors involve neural pathways having similar pharmacological characteristics. The inability of clonidine pretreatment to affect atropine-promoted locomotion and rearing is consistent with the concept that the neural pathways mediating these behaviors may be distinguished.

Clonidine shares many autonomic effects with atropine including the development of dry mouth, mydriasis and urinary retention [16]. At clinically employed dose levels, clonidine does not produce muscarinic receptor blockade, but does inhibit postganglionic cholinergic neurons [6, 8, 18]. Experiments in this laboratory have confirmed this presynaptic inhibitory action of clonidine on central cholinergic neurons [2–4]. It is possible that this difference in site of cholinergic antagonism for clonidine and atropine (presynaptic versus postsynaptic) explains the differences in their behavior promoting actions and mutual inhibition. More likely, these differences may be explained by clonidine's ability to interact with several different neurotransmitter systems. Clonidine has been examined as a potential therapeutic agent in several psychological and neurological disorders and has been found to exhibit significant activity in several of them [10,12]. The neurochemical basis for the inhibitory action of clonidine on atropine induced stereotypy may provide insights into the psychotropic effects of clonidine and thus warrants further investigation.

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