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Prevention of Reserpine Rigidity by Alpha-2 Adrenergic Antagonists

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WAGNER, B. H. AND R. J. ANDERSON. *Prevention of reserpine rigidity by alpha-2 adrenergic antagonists.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 731–735, 1982.—Since alpha adrenergic antagonists are known to protect rats from the extrapyramidal effects of reserpine, the purpose of this study was to examine the relative contribution of alpha-2 receptors in modifying the reserpine-induced syndrome. Rats were pretreated with either clonidine, yohimbine, phentolamine, methysergide or SKF-7265. Thirty minutes later they were given reserpine (20 mg/kg) and evaluated using eleven categories of behavioral responses for three hours. Yohimbine, an alpha-2 antagonist, was the most effective agent in protecting against the reserpine effects. Phentolamine and SKF-7265, which block both alpha-1 and alpha-2 receptors, were also effective. Clonidine, an alpha-2 agonist, and methysergide a serotonin antagonist, were not. In all cases the alpha blocking drugs prevented the motor responses but did not alter the autonomic responses induced by reserpine. The results show not only the efficacy of alpha adrenergic antagonists in protecting against reserpine rigidity but more importantly that the blockade of alpha-2 receptors may be the functionally important action. These results are consistent with the view that some descending motor pathways are controlled by an adrenergic mechanism and suggest that alpha-2 receptors are an important component.

Alpha adrenergic antagonists Extrapyramidal effects Reserpine Alpha-2 receptors Motor responses Autonomic responses

IN addition to its value as an antipsychotic and antihypertensive agent, reserpine induces a repertoire of behavioral effects which have been used as animal models of several neurologic disorders. Large, acute doses of reserpine given to rats induce the release and depletion of dopamine, norepinephrine and serotonin throughout the neuroaxis and result in a spectrum of extrapyramidal and autonomic effects.

The reserpinized rat has been more widely recognized as an animal model for screening antiparkinson drugs [8, 10, 11, 19]. Like Parkinson's disease, reserpine produces tremor, rigidity and loss of spontaneous motor activity [12, 16, 21] which result from deficient activity of dopamine receptors in the striatum [9]. These extrapyramidal effects are reversed by a number of antiparkinson drugs including antimuscarinic agents [11,16], dopamine agonists and DOPA [10,12], which presumably also act within the striatum to restore the dopaminergic balance [17]. Interestingly, antipsychotics such as chlorpromazine and trifluoperazine [17] also reduce reserpine rigidity. However, these drugs probably act at a motor control locus separate from the striatum and via blockade of alpha adrenergic receptors.

Alpha adrenergic receptors are widely recognized as an important mediator of motor control. Several descending noradrenergic pathways involved with motor control have been identified [4,5], and the motor neuron hyperexcitability induced by a variety of central lesions is effectively suppressed by alpha adrenergic antagonists [13, 14, 15]. The role of alpha receptors was also demonstrated in a study in which we showed that chlorpromazine and 10-(2-dimethylaminoethyl) acridan (SKF-7265), two drugs with significant alpha blocking potency, protected rats from reserpine rigidity whereas promethazine, a phenothiazine without adrenergic activity, did not [20]. However, no previous study of motor control pathways has made a distinction between the contribution of alpha-1 and alpha-2 receptors, and most of the adrenergic drugs used in these studies have affinity for both receptor types. The purpose of our study therefore was to examine more selectively the role of alpha-2 receptors in modifying the reserpine syndrome using clonidine, an alpha-2 agonist, and yohimbine, an alpha-2 antagonist, as the test compounds.

METHOD

Male Sprague-Dawley rats (218–350 grams) were coded in groups of five and pretreated with intraperitoneal injections of one of the test drugs (clonidine: 0.04, 0.2, and 1.0 mg/kg; yohimbine: 1.25, 2.5, and 5.0 mg/kg; SKF 7265: 36, 72, and 144 mg/kg; phentolamine: 1.0 mg/kg), saline or saline acidified with HCl. To check the possible involvement of serotonin in the reserpine syndrome, three additional animals were pretreated with the serotonin antagonist, methysergide (1.0 mg/kg). All drugs were dissolved in 0.9% saline except SKF-7265, which was soluble in saline acidified with HCl to a pH of 2.0. All solutions were given in a volume less than 1.5 ml. Twenty minutes later an observer, who was unaware of the drug or dose, evaluated each rat for

TABLE 1
CHARACTERISTICS OF THE RESERPINE SYNDROME BY CATEGORY

Observation	Score	Definition
Body Tremor	0	Absent
	1	Present
Bursting	0	Absent
	1	Present
Exploratory Behavior	0	Present
	1	Absent
Facial Twitch	0	Absent
	1	Present
Provoked Motor Activity	0	Present
	1	Absent
Ptosis	0	Eyelids Open
	1	Eyelids Partially Closed
	2	Eyelids Fully Closed
Posture	0	Any Posture Other Than Hunched
	1	Partially Hunched
	2	Fully Hunched
Rigidity	0	No Rigidity
	1	Moderately Rigid
	2	Severely Rigid
Tail Muscle Tone	0	Normal
	1	Greater Than Normal Yet Not Rigid
	2	Rigid With No Kinking of Tail
	3	Rigid With Kinking of Tail
Hindlimbs	0	Extend Laterally
	1	Flexed But Not Crossed
	2	Flexed and Crossed
Global	0	Normal
	1	Moderately Affected
	2	Severely Affected

signs of abnormal behavior. Thirty minutes following the test drug injection, reserpine (20 mg/kg) was administered intraperitoneally. The rats were then evaluated by the unbiased observer for evidence of the reserpine syndrome at twenty minute intervals for a period of three hours.

The extrapyramidal and autonomic characteristics of the reserpine syndrome have previously been described [7, 20, 21]. In the present experiments these were categorized and quantitated using the scoring system described in Table 1. The first five characteristics were scored on a quantal two-point scale. The remaining categories were graded as to severity. Since there was an indication that the effects of the high doses of clonidine and yohimbine were contributing to or disrupting evaluation of the reserpine syndrome, additional animals were dosed with these agents, but not with reserpine, and then evaluated over the same time course. For each of the eleven categories, the scores of the nine observation periods were summed, thus giving category scores for each rat which represented a combined index of the magnitude and duration of the reserpine effect. The data are reported in terms of mean score per animal and listed by individual category for each drug treatment.

In high concentrations, clonidine can activate alpha-1 adrenergic receptors as well as alpha-2 receptors. To check for this effect, the ability of clonidine to increase blood pressure (via alpha-1 mediated vasoconstriction) was evaluated. Yohimbine, which might also affect blood pressure in high doses due to blockade of alpha-1-adrenergic receptors, was also tested. Forty-five minutes after intraperitoneal doses of yohimbine (1.25, 2.5 or 5.0 mg/kg) or clonidine (0.04, 0.2 or 1.0 mg/kg) systolic blood pressure was measured indirectly in the conscious rat using tail plethysmography. The average of three measurements was compared to the average blood pressure from the same six animals before drug treatment. Because blood pressure could not be recorded from the tail after the two higher doses of clonidine, two additional rats were used to measure blood pressure directly. An indwelling catheter was placed in the carotid artery under ether anesthesia. After surgery the anesthesia was discontinued and the rat was restrained. Blood pressure was monitored continuously with a Statham P23Db blood pressure transducer which was connected to a Beckman amplifier recorder system that had been calibrated against a mercury manometer. Recordings were made before and after intraperitoneal clonidine administration.

The ability of each test agent to modify the reserpine syndrome was analyzed using the Kruskal-Wallis single factor analysis of variance by ranks. SKF-7265 treated animals were compared to the acidified saline controls. All other drug treatment groups were compared to neutral saline

TABLE 2

MEAN SCORES OF THE RESERPINE SYNDROME BY CATEGORY AFTER PRETREATMENT WITH YOHIMBINE

		Yohimbine		
Reserpine Effect	Saline	1.25 mg/kg	2.5 mg/kg	5.0 mg/kg
Body Tremor	4.0	0*	1.2*	0*
Rigidity	13.0	6.8*	6.0	3.5*
Posture	7.4	4.0*	3.4*	2.3*
Tail Muscle Tone	18.8	10.8*	11.8*	6.2*
Facial Twitch	6.8	4.4*	4.0*	3.2
Hindlimbs	8.6	6.6	7.6	8.2
Provoked Motor Activity	7.6	7.0	8.8	6.8
Ptosis	11.4	10.6	8.6	9.9
Exploratory Activity	7.8	7.4	8.4	6.2
Bursting	0	0	0	0
Global	14	11.2	12.6	10.8
Number of Subjects	5	5	5	5

*$p<0.05$ compared with the saline pretreated control.

TABLE 3

MEAN SCORES OF THE RESERPINE SYNDROME BY CATEGORY AFTER PRETREATMENT WITH SKF-7265

		SKF-7265		
Reserpine Effect	Acidified Saline	36 mg/kg	72 mg/kg	144 mg/kg
Body Tremor	7.7	3.2*	2.2	0*
Rigidity	7.8	10.8	8.2	3.4
Posture	6.0	6.5	5.6	2.1
Tail Muscle Tone	17.6	16	13.6	8.2
Facial Twitch	7.4	4.6	5.4	3.8
Hindlimbs	5.8	6.8	6.8	5.4
Provoked Motor Activity	5.4	7.6	7.6	6.4
Ptosis	9.6	10.6	8.8	9.8
Exploratory Activity	6.0	7.8	8.2	7.4
Bursting	0	0.4	0.2	0.4
Global	10.2	12.2	11.0	11.8
Number of Subjects	5	5	5	5

*$p<0.05$ compared with the acidified saline pretreated control.

treated animals. Those drugs which produced a significant response according to the Kruskal-Wallis test were further analyzed to determine significant differences between control responses and the responses of individual doses using the non-parametric multiple comparison test [24]. Values with $p<0.05$ were considered statistically significant.

RESULTS

Reserpine induced a body tremor, rigidity and akinesia resembling Parkinsonism. In animals pretreated with only saline the reserpine syndrome was prominent for 2 hours and began to subside three hours after reserpine administration. The animals treated with acidified saline had more variable responses than the neutral saline group.

Yohimbine, phentolamine and SKF-7265 each reversed the magnitude of the reserpine syndrome, particularly those features considered to be extrapyramidal signs. Table 2 shows the effect of each dose of yohimbine on the reserpine syndrome by category. The 1.25 mg/kg dose of yohimbine was maximally effective and the higher doses provided no further protection from the reserpine syndrome. SKF-7265 also appeared to reduce the severity of the reserpine syndrome, but this was only statistically significant at the 36 and 144 mg/kg doses for tremor, as shown in Table 3. The greater variability in the responses of the vehicle controls used in

TABLE 4

MEAN SCORES OF THE RESERPINE SYNDROME BY CATEGORY AFTER PRETREATMENT WITH CLONIDINE, PHENTOLAMINE, OR METHYSERGIDE

Reserpine Effect	Saline	Phentolamine (1 mg/kg)	Methysergide (1 mg/kg)	Clonidine (0.04 mg/kg)
Body Tremor	4.0	2.2*	3.3	6.2*
Rigidity	13.0	6.4*	13.7	12.6
Posture	7.4	4.8*	7.5	7.9
Tail Muscle Tone	18.8	14.8	19.7	18.2
Facial Twitch	6.8	6.4	6.3	6.4
Hindlimbs	8.6	7.0	6.3	4.4
Provoked Motor Activity	7.6	7.0	8.0	7.8
Ptosis	11.4	12.0	14.7	2.0*
Exploratory Activity	7.8	7.6	8.0	8.2
Bursting	0	0.4	0	1.8
Global	14.0	9.6	13.3	10.4
Number of Subjects	5	5	3	5

*$p<0.05$ compared with saline pretreated control.

this portion of the study may account for the lack of statistical significance. Phentolamine (1 mg/kg), a non-selective alpha adrenergic antagonist, was effective against the extrapyramidal effects of reserpine as shown in Table 4. On the other hand, methysergide, a serotonin antagonist, and the 0.04 mg/kg dose of clonidine did not alter the reserpine syndrome, except for body tremor which was increased.

The 0.2 and 1.0 mg/kg doses of clonidine did modify the reserpine syndrome significantly. However, these doses also produced a number of additional side effects which were not present in the control animals, including exophthalmos, chromodacryorrhea and piloerection, making evaluation of the reserpine syndrome difficult.

Since clonidine in high doses will activate alpha-1 receptors, the effect of clonidine on systolic blood pressure was measured to determine the extent of alpha-1 activation. The 0.04 mg/kg dose of clonidine did not alter blood pressure. However, the higher doses produced a profound vasoconstriction which precluded accurate measurement of blood pressure from the tail artery. Using an indwelling catheter in unanesthetized rats, the higher doses of clonidine increased blood pressure 25 mm Hg. Yohimbine did not alter blood pressure measured by the tail plethysmograph method. The other test drugs produced no side effects except occasional muscle relaxation, which was characterized as a flattened posture, at the higher doses.

DISCUSSION

These results show that the reserpine syndrome can be antagonized by blockade of alpha-2 receptors but that alpha-2 activation does not exaggerate the effects of reserpine. All three drugs with alpha-2 blocking potency (yohimbine, SKF-7265 and phentolamine) reversed the syndrome in the approximate order of their affinity for alpha-2 receptors. Although yohimbine, the most selective alpha-2 antagonist, also has dopamine blocking activity [18], the anti-rigidity effect is more likely due to alpha receptor blockade since phentolamine and SKF-7265, alpha blocking drugs with little affinity for dopamine receptors [22], were also effective against the reserpine syndrome. In addition, specific dopamine antagonists such as haloperidol do not reduce the rigidity induced by reserpine [10].

The alpha blocking drugs protected animals from the extrapyramidal aspects of the reserpine syndrome (rigidity, tremor, posture) but did not alter the intensity of the other effects induced by reserpine. This is consistent with other reports [7, 16, 20] which have drawn a distinction between the somatic and autonomic effects of reserpine. The extrapyramidal effects of reserpine can be blocked by dopaminergic agonists or alpha adrenergic antagonists. However, these drugs are ineffective against reserpine induced ptosis, which is reversed by the tricyclic antidepressants [7]. These two aspects of the reserpine syndrome, therefore, appear to be separate both as to origin and prevention.

Alpha adrenergic blockade has been implicated as an important mediator of motor control by a number of investigators [1, 13, 15, 23]. Exaggerated muscle tone induced by multiple sclerosis [13], decerebrate rigidity [6,15], cerebral palsy and stroke [3,23] have been reduced by alpha adrenergic blockade. However, no attention has been paid to the contribution of alpha-2 receptors in this process. The present results showing yohimbine to be the most effective agent against all the somatic effects of reserpine, suggest that alpha-2 receptor blockade may be the more important effect. However, the site of this drug action within the motor control pathway is still obscure.

Although alpha-2 receptor blockade is effective in preventing the extrapyramidal signs of reserpine, activation of alpha-2 receptors does not induce nor enhance the expression of the reserpine syndrome. Clonidine did not modify the reserpine syndrome when given in a dose chosen to be selective for alpha-2 receptor activation. However, the larger doses of clonidine produced a number of side effects indicative of alpha-1 activation (hypertension, piloerection, exophthalmos) which complicated evaluation of the reserpine syndrome. The inability of clonidine to exacerbate the reserpine syndrome may relate to the recent observation that the distribution of specific alpha-2 binding sites within the central nervous system is different for yohimbine and clonidine [2], suggesting subpopulations of alpha-2 receptors.

Our results suggest that alpha-2 blockade is an important factor in suppressing the reserpine syndrome. Furthermore, it may be responsible for a selective antirigidity effect rather than generalized sedation. The muscle weakness and sedative effects, which are characteristic of chlorpromazine [20] in antirigidity doses, were very infrequent with both yohimbine (present experiments) and SKF-7265 [20]. The latter two drugs have a much greater affinity for alpha-2 receptors than does chlorpromazine [22]. Because of its relatively low affinity for alpha-2 receptors, large doses of chlorpromazine were needed in order to provide sufficient alpha-2 blockade to protect against the reserpine syndrome. In the process chlorpromazine would be expected to produce a number of other effects, including sedation, due to actions on dopamine, alpha-1 and serotonin receptors, for which the drug has a greater affinity. Our results suggest that future studies should recognize the contribution of alpha-2 adrenergic receptors in modifying motor output.

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