Prevention by Antidepressant Drugs of Reserpine Effect on Pyridine Nucleotide Metabolism

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SUMMARY

A variety of structurally unrelated antidepressant drugs, including both monoamine oxidase (MAO)-inhibitors and non-MAO-inhibitors, prevent the effect of reserpine on the nicotinamide-induced increase in NAD. A single injection of imipramine or iproniazid was effective when given at any time up to several days prior to reserpine. In general, compounds other than antidepressants did not prevent the reserpine effect on NAD metabolism. The ability to block the effect of reserpine on NAD metabolism correlated well with the ability to block the sedative effect of reserpine. The antidepressants did not prevent the effect of chlorpromazine on NAD level, in agreement with their lack of effect on the sedative action of chlorpromazine.

INTRODUCTION

Reserpine prolongs the transient increase of liver NAD *in vivo* which follows the administration of nicotinamide (1, 2). The mechanism by which reserpine elicits this biochemical effect is not yet known. Since, as found by Brodie and his colleagues (e.g., 3–5), many pharmacologic effects of reserpine can be prevented or reversed by antidepressant drugs, it was of interest to determine whether antidepressants might also reverse the effect of reserpine on this biochemical phenomenon. In the present study, it was found that both types of antidepressant agents, MAO-inhibitors and non-MAO-inhibitors, do prevent the effect of reserpine on pyridine nucleotide metabolism.

METHODS

Mature female Wistar rats (Royal Hart Farms) weighing 150–160 g were used. Animals were killed by cervical fracture. Liver NAD was determined by a modification of the Racker alcohol dehydrogenase method (6, 7), as described previously (8).

RESULTS

The effect of reserpine on the nicotinamide-induced increase of liver NAD is illustrated in Fig. 1. In the presence of reserpine, the increase in NAD is less marked 6 and 12 hr after nicotinamide administration, but the elevation of NAD is prolonged by the tranquilizer. A single injection of iproniazid 18 hr prior to the administration of reserpine prevented the effect of the tranquilizer: the NAD response in animals given both iproniazid and reserpine prior to nicotinamide was similar to the response in animals given nicotinamide alone. Imipramine gave results similar to those shown for iproniazid. For all subsequent studies, the concentration of NAD was determined 24 hr after administration of nicotinamide.

A variety of structurally dissimilar antidepressants, including both MAO-inhibitors and non-MAO-inhibitors, were found capable of preventing the NAD response to reserpine. The effect of varying doses of imipramine, demethylimipramine, and iproniazid on the reserpine-induced eleva-

TABLE 1

Reversal by antidepressants of reserpine effect on liver NAD and on sedation: variation of dose of antidepressant

All animals used for determination of NAD were given 500 mg/kg of nicotinamide, i.p., and killed 24 hr later. Antidepressant agents were administered 20 hr, and reserpine 2 hr, prior to nicotinamide. Each NAD value represents the mean ± standard error for 6 animals. For determination of sedation, a separate group of animals not given nicotinamide was used. The presence or absence of sedation was observed for several hours after reserpine administration.

Antidepressant	Dose of antidepressant (mg/kg, i.p.)	Dose of reserpine (mg/kg, s.c.)	Effect on NAD: NAD (μg/g wet wt)	Effect on NAD: Increase in NAD (μg/g wet wt)	Effect on NAD: Per cent inhibition[a]	Sedation
None	—	—	651 ± 99	—	—	No
None	—	5	1436 ± 296	785	—	Yes
Imipramine	10	5	1463 ± 155	812	−4	Yes
	25	5	641 ± 71	−10	+101	No
	50	5	558 ± 43	−93	+112	No
Demethylimipramine	10	5	1299 ± 226	648	+17	Yes
	25	5	582 ± 11	−69	+109	No
	50	5	589 ± 57	−62	+108	No
Iproniazid	10	5	1657 ± 178	1006	−28	Yes
	25	5	572 ± 85	−79	+110	No
	50	5	507 ± 31	−144	+118	No

[a] Per cent inhibition by the antidepressant agents of the reserpine effect on NAD was calculated from the formula:

$$100\left(1 - \frac{\text{increase in NAD in presence of reserpine + antidepressant}}{\text{increase in NAD in presence of reserpine only}}\right)$$

tion of NAD and sedation are shown in Table 1. All three compounds reversed both the biochemical and the pharmacologic effects of reserpine when administered in doses of 25 and 50 mg/kg. Neither effect of reserpine was reversed by a 10 mg/kg dose of these antidepressants, with the possible exception of demethylimipramine, which did show a slight inhibition of the NAD effect at this dose.

The effect of administration of antidepressants simultaneously with or at various times prior to reserpine is shown in Table 2. A single injection of either imipramine or iproniazid inhibited the reserpine effect on NAD when given at any time up to several days prior to reserpine. Imipramine showed a more rapid onset of action than iproniazid. On the other hand, iproniazid was effective for a longer period of time than imipramine; iproniazid caused greater than 50% inhibition as late as 5 days following administration of a single dose. Imipramine and iproniazid did not prevent the action of reserpine when given 3 or 6 hr after the tranquilizer.

A variety of compounds belonging to various pharmacological categories were tested for effectiveness in preventing the reserpine-induced increase in NAD (Table 3). With the exception of pentobarbital, complete or near complete inhibition was observed only with compounds which belong to the antidepressant class. It is noteworthy that isoniazid, which is structurally similar to iproniazid, but which has little or no antidepressant activity and is not an MAO-inhibitor, was unable to reverse the reserpine effect on NAD.

As in the case of reserpine, chlorpromazine prolongs the nicotinamide-induced increase in liver NAD (1, 2). It was found in

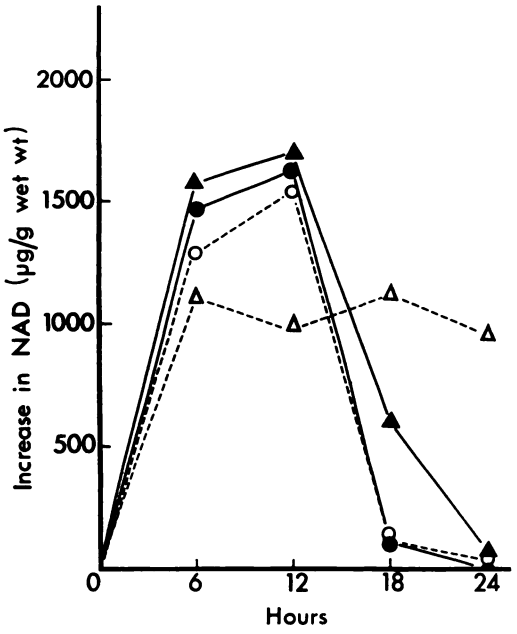

FIG. 1. *Reversal by iproniazid of reserpine effect on liver NAD*

The increase in rat liver NAD is shown as a function of time following the administration of nicotinamide (500 mg/kg, i.p.). The increase in NAD was obtained by subtracting the endogenous value of 430 μg/g of NAD, found in liver extracts from control rats. Iproniazid (50 mg/kg, i.p.) was administered 20 hr prior and reserpine (5 mg/kg, s.c.) 2 hr prior to nicotinamide. Each value represents the mean for 6 animals. Nicotinamide alone, ○---○; reserpine plus nicotinamide, △---△; iproniazid plus reserpine plus nicotinamide, ▲---▲; iproniazid plus nicotinamide, ●——●.

the present investigation that the antidepressants do not reverse the effect of chlorpromazine on NAD. In fact, in each of several experiments the NAD level was slightly higher in the presence of antidepressant drug plus chlorpromazine than in the presence of chlorpromazine alone. A typical experiment is shown in Table 4. Moreover, the antidepressant agents tested had no apparent effect on the sedation induced by chlorpromazine administration.

DISCUSSION

The effects of psychoactive drugs on NAD level described in the present work were obtained in liver. Reserpine does cause

TABLE 2

Reversal by antidepressants of reserpine effect on liver NAD: variation of time between antidepressant and reserpine administration

All animals were given nicotinamide (500 mg/kg, i.p.), and NAD was determined 24 hr later. Reserpine (5 mg/kg, s.c.) was administered 2 hr prior to nicotinamide. Antidepressant agents (50 mg/kg, i.p.) were administered at the indicated intervals of time prior to reserpine. Per cent inhibition by the antidepressant agents of the reserpine effect on NAD was calculated as described in Table 1. Each value represents the mean of results obtained in 2–7 experiments, in each of which 6 animals were used per group.

Time before reserpine	Per cent inhibition by antidepressant	
	Imipramine	Iproniazid
0 hr	68	12
3 hr	84	75
6 hr	84	66
12 hr	98	83
18 hr	104	104
24 hr	104	91
2 days	102	99
3 days	33	79
4 days	30	65
5 days	—	58

an effect in brain similar to, but much smaller than, that observed in liver and, therefore, an extremely large number of animals would have been needed to obtain statistically significant data on the effect of antidepressants on brain levels of NAD.

Of the seven antidepressant drugs tested in the present study, all but amitriptyline strongly inhibited the effect of reserpine on NAD. It is of interest in this connection that amitriptyline was the only one of several antidepressant drugs which failed to prevent the reserpine-like behavioral syndrome produced by Ro 4-1284 (4). In addition, amitriptyline was the only one of these antidepressants which failed to potentiate amphetamine-induced hyperthermia in rats (9).

Of the eighteen nonantidepressant compounds studied, only one, pentobarbital, strongly inhibited the reserpine effect on NAD. Several other compounds caused a weak inhibition. Properties unrelated to

TABLE 3

Reversal by antidepressants of reserpine effect on liver NAD: specificity of reversing agents

All animals were given nicotinamide (500 mg/kg, i.p.), and NAD was determined 24 hr later. Test compounds were administered 20 hr, and reserpine (5 mg/kg, s.c.) 2 hr, prior to nicotinamide. Test compounds were administered intraperitoneally in doses equimolar to 50 mg imipramine per kilogram. Individual determinations of NAD were made in at least 6 animals for each compound tested. Per cent inhibition by the test compounds of the reserpine effect on NAD was calculated as described in Table 1.

Class	Compound	Per cent inhibition
Antidepressant: non-MAO-inhibitor	Imipramine	102
	Demethylimipramine	91
	Amitriptyline	8
	Demethylamitriptyline	68
Antidepressant: MAO-inhibitor	Iproniazid	103
	Tranylcypromine	90
	Pargyline	95
CNS stimulant	Amphetamine	24
	Caffeine	−25
Anticonvulsant	Diphenylhydantoin	8
Sedative	Meprobamate	31
	Hexobarbital	17
	Pentobarbital	90
Narcotic analgesic	Morphine	42
Muscle relaxant	Zoxazolamine	19
Antihypertensive	Hydralazine	−7
Anti-inflammatory analgesic	Phenylbutazone	43
	Aminopyrine	23
Antimalarial	Quinacrine	8
Antitubercular	Isoniazid	−1
Anticholinergic	Atropine	9
	Benactazine	17
Parasympathomimetic	Methacholine	24
Antihistamine	Chlorpheniramine	46
	Tripelennamine	16

TABLE 4

Lack of reversal by antidepressants of chlorpromazine effect on liver NAD

All animals were given nicotinamide (500 mg/kg, i.p.), and NAD was determined 24 hr later. Antidepressants (50 mg/kg, i.p.) were administered 20 hr prior and chlorpromazine 2 hr prior to nicotinamide. Each NAD value represents the mean ± standard error for the number of animals indicated in parentheses.

Antidepressant	Dose of chlorpromazine (mg/kg, i.p.)	NAD (μg/g wet wt)
—	—	480 ± 42 (5)
—	25	1967 ± 249 (9)
Imipramine	25	2265 ± 208 (8)
Demethylimipramine	25	2418 ± 265 (7)
Iproniazid	25	2280 ± 415 (5)

antidepressant potency could be responsible for the ability of certain compounds to inhibit the reserpine effect. For instance, pentobarbital and phenylbutazone are known to be effective in inducing the formation of the microsomal enzyme system involved in drug catabolism, and it is possible that their effectiveness in the present investigation resulted from an increased rate of breakdown of the administered reserpine. It would be informative to investigate the effect on reserpine metabolism of those compounds capable of reversing the effects of reserpine.

The partial effectiveness of chlorpheniramine, an antihistamine, in reversing the effect of reserpine on NAD, is in accordance with the observations of Sigg *et al.* (10) of the effectiveness of some antihis-

tamines in preventing behavioral effects of reserpine.

The sedative effects of reserpine can be prevented by cold stress (2, 11, 12) as well as by either the iproniazid (MAO-inhibitor) class (3) or the imipramine (non-MAO-inhibitor) class (4, 5) of antidepressant drugs. The ability of cold stress (2) and of treatment with either of these two classes of antidepressant drugs (present investigation) to prevent the effect of reserpine on NAD level thus correlates with the ability of these procedures to prevent reserpine sedation. Despite these and other (2) correlations between the effects of the psychoactive drugs on behavior and on NAD level, it seems highly unlikely that any causal relationship exists between the two effects.

For a number of reasons, it seems possible that the parallel effects of the psychoactive drugs on behavior and on NAD level result from some common primary action on the hypothalamus. It is generally accepted that drugs affecting behavior do so by modification of hypothalamic function. The effects of the psychoactive drugs on NAD level can also be explained in terms of a primary action on the hypothalamus. This suggestion is based, in part, on the fact that cold stress and administration of reserpine cause pronounced changes in endocrine function. In addition, recent evidence indicates that NAD metabolism is greatly influenced by the endocrine system: thus removal of the adrenal or thyroid glands increases NAD level in nicotinamide-treated rats, whereas adrenocorticoids and thyroxine have the opposite effect (13). Therefore, it is postulated that the psychoactive drugs, by affecting that part of the hypothalamus which regulates anterior pituitary activity, indirectly affect the function of the target endocrine glands and, thereby, NAD metabolism. The effect of various psychoactive drugs on peripheral hormone levels is now being investigated under conditions in which alterations in NAD level and in sedation occur.

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