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DIFFERENCES BETWEEN THE EFFECTS OF ACUTE AND LONG-TERM TREATMENT WITH DESMETHYLIMIPRAMINE ON RESERPINE-INDUCED RELEASE OF AMINES FROM RAT BRAIN

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Abstract—A single injection of the tricyclic anti-depressant drug desmethylimipramine (DMI) caused a small, transient and consistent retardation of reserpine-induced release of rat brain norepinephrine but not of dopamine and serotonin. In contrast, long-term treatment with desmethylimipramine enhanced the release of rat brain norepinephrine, dopamine and serotonin after reserpine. It was found that the older animals were less sensitive than the younger animals to the brain amine depletion caused by reserpine. However, the interaction between DMI and reserpine was not affected by the age of the animals; the antagonism between acute DMI and reserpine occurred in both young and old rats.

The most well-known effect of the tricyclic anti-depressant drug desmethylimipramine (DMI), the inhibition of uptake of norepinephrine (NE) at the neuronal membrane in sympathetically innervated tissues, occurs after a single injection of the drug [1–4]. Evidence has now accumulated which indicates that a single injection of DMI acts also in the intraneuronal mechanism which concentrates NE in the storage vesicle [5–14]. Since the clinical efficacy of the tricyclic anti-depressants appears only after long-term treatment, it is important to know the neurochemical consequences of chronic administration of these drugs. Differences in turnover and content of brain NE have been reported between the effects of acute and chronic administration of DMI and other tricyclic anti-depressant drugs [15–17]. In the present studies, we have tried to test the possibility that DMI given over a long period has a different intraneuronal action than does acutely administered DMI. Thus, we have compared reserpine-induced release of brain NE in rats which were treated previously with DMI on an acute or a chronic schedule. Reserpine is believed to cause intraneuronal release of amines because of its interference with amine storage. We have also measured the retention of brain dopamine and serotonin after reserpine administration in animals treated acutely and chronically with DMI to establish whether the effect of DMI on intraneuronal storage sites is confined to those containing NE. We found no effect of age of the animals on the interaction between DMI and reserpine but we did see that sensitivity to reserpine decreases with age.

METHODS AND MATERIALS

Male Sprague–Dawley rats (180–200 g) received either a single intraperitoneal injection of desmethylimipramine hydrochloride (10 mg/kg) or were treated for 4 weeks with DMI dissolved in the drinking water. The volume of drinking water consumed by a group of rats housed together was measured daily and the dose of DMI dissolved in the water was adjusted accordingly. We found that each animal drank about 20 ml water/day; the concentration of DMI in the drinking water was adjusted to provide a dose of approximately 20 mg/kg/day. Fresh solutions of DMI were made twice each day. A group of matched control animals was included in every experiment. Control animals for the group which received orally administered DMI were given tap water and their cages were kept in the same rack as the DMI-treated animals throughout the period of drug administration. Control animals for the experiments using acutely administered DMI were given an intraperitoneal injection of saline.

One hr after the single injection of DMI or saline, animals were injected intraperitoneally with reserpine (2 mg/kg). The reserpine (10 mg) was dissolved in 1 ml of 20% ascorbic acid and then diluted with water for injection. Control animals were injected with the appropriate dilution of ascorbic acid. Chronically treated animals and their controls were given an intraperitoneal injection of reserpine (2 mg/kg) or vehicle approximately 3 hr after the drinking water had been removed from the cages. In a few experiments, the purpose was to compare the effects of reserpine on amine depletion between 50-day-old and 80-day-old rats. The older animals were purchased 40 days before the younger ones. The two groups were housed together for 1 week prior to the experiment. The older and younger animals were injected and killed on the same day using a staggered schedule.

The animals were killed 45 min after the reserpine injection unless stated otherwise. The animals were decapitated and their brains were removed. Norepinephrine extracted from the brains was assayed according to the procedure of Anton and Sayre [18]. Dopamine was assayed fluorimetrically after separation on Alumina according to the procedure of Carlsson and Waldeck [19]. Serotonin was collected in the effluent from the Alumina columns. An aliquot of this was used subsequently for the separation and

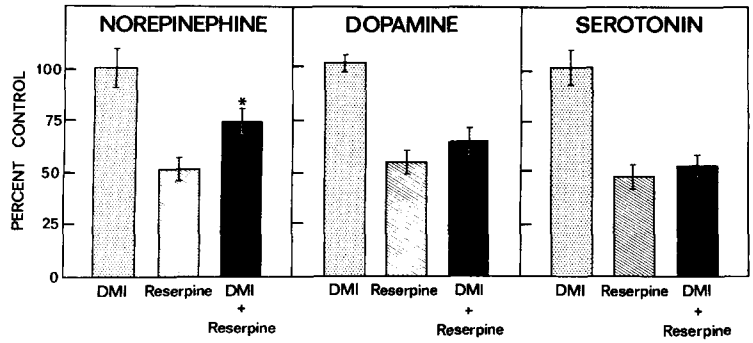

Fig. 1. Effect of a single intraperitoneal (i.p.) injection of DMI (10 mg/kg) on reserpine (2 mg/kg, i.p.)-induced release of rat brain norepinephrine (NE), dopamine (DA) and serotonin (5-HT). The reserpine was injected 1 hr after the DMI injection. The animals were killed 45 min after reserpine. The results are expressed as per cents of the mean concentration of amine in the matched control group. The concentration of NE in the control group was 291 ± 24 ng/g, that of DA was 619 ± 38 ng/g and that of 5-HT was 186 ± 16 ng/g. Each control and experimental result represents the mean ± standard error of the mean of 12–16 observations. The asterisk (*) indicates $P < 0.01$ compared to reserpine-treated group.

assay of serotonin according to the procedure described by Bertler [20] and modified by Anden and Magnusson [21]. Large amounts of DMI (up to 10 mg) added to homogenates of rat brain did not interfere with any of the amine assays. The biochemical data have not been corrected for recovery of amine. The average recoveries were 80 per cent for norepinephrine, 78 per cent for dopamine and 55 per cent for serotonin. In order to combine the results from various separate experiments which examined the effects of acute or chronic DMI on reserpine-induced release of amine, the results were expressed as per cents of the matched control mean values. The experimental results were evaluated statistically by the Student *t*-test.

Desmethylimipramine hydrochloride was a gift from USV Pharmaceutical Corp. Reserpine was purchased from Regis Chemical Co.

RESULTS

Effect of acute desmethylimipramine on reserpine-induced release of norepinephrine, dopamine and serotonin. A single dose of DMI (10 mg/kg) significantly retarded the reserpine-induced release of NE when animals were killed 45 min after the reserpine injection (Fig. 1). The reserpine-induced release of neither DA nor 5-HT was significantly affected by prior injection of DMI (Fig. 1). Additional experiments were done to see if DMI does counteract significantly the dopamine depletion by reserpine with a different time course than seen with NE. As seen in Fig. 2, the "saving" of NE in DMI-treated animals was seen 15, 45, 60 and 120 min after reserpine treatment in contrast to DA which was not significantly conserved at any of these time periods. The conservation of NE did not persist, since no NE could be measured in brains of animals treated with DMI 17 hr after the reserpine injection. When animals were given a higher dose of DMI (25 mg/kg), norepinephrine was still significantly conserved 45 min after the reserpine injection, whereas dopamine and serotonin were not.

Effects of long-term treatment with desmethylimipramine on reserpine-induced release of norepinephrine, dopamine and serotonin. Long-term treatment with DMI reduced the NE content of brain by approximately 20 per cent (Fig. 3). The loss of NE caused by reserpine in animals chronically treated with DMI was greater than that caused by reserpine in untreated

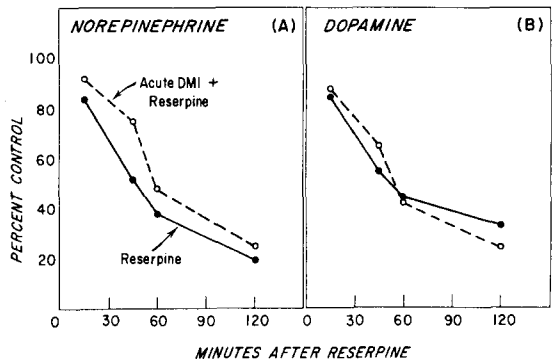

Fig. 2. (A) Fall of NE content from brains of rats which received one intraperitoneal injection of DMI (10 mg/kg) and from brains of saline-injected rats at various intervals after an intraperitoneal injection of reserpine (2 mg/kg). Rats were given the reserpine injection 1 hr after the DMI injection. The results are expressed as per cents of the mean NE concentration in the matched control group. Each point is the mean value for 9–16 animals. The mean and standard error for each time point represented for reserpine-treated animals, starting with those killed 15 min after reserpine, were: 84 ± 2, 52 ± 5, 38 ± 2 and 19 ± 3 per cent. The mean and standard error for each time point represented for the DMI plus reserpine-treated animals, starting with those killed 15 min after reserpine, were: 92 ± 3, 75 ± 6, 48 ± 2 and 25 ± 3 per cent. (B) Fall of dopamine content from brains of rats which received one injection of DMI (10 mg/kg, i.p.) and from brains of saline-injected rats at various intervals after an injection of reserpine (2 mg/kg, i.p.). Rats were given the reserpine injection 1 hr after the DMI injection. Results are expressed as per cents of the mean dopamine concentration in the matched control group. Each point is the mean value for 5–16 animals. The mean and standard error for each time point represented for reserpine-treated animals, starting with those killed 15 min after reserpine, were: 86 ± 3, 55 ± 6, 44 ± 4 and 33 ± 14 per cent. The mean and standard error for each time point represented for the DMI plus reserpine-treated animals, starting with those killed 15 min after reserpine, were: 88 ± 3, 65 ± 7, 42 ± 5 and 26 ± 7 per cent.

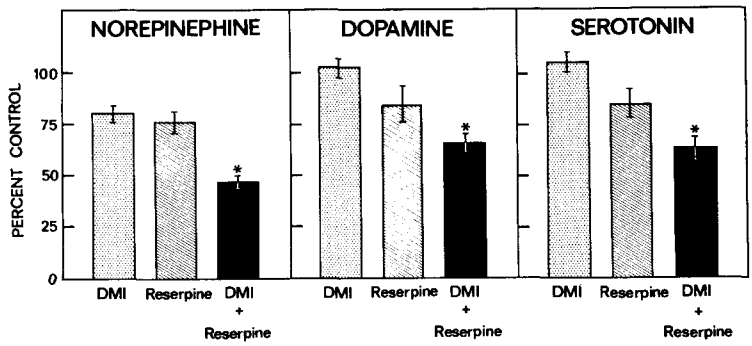

Fig. 3. Effect of chronic administration of desmethylimipramine (DMI) on reserpine (2 mg/kg, i.p.)-induced release of rat brain norepinephrine (NE), dopamine (DA) and serotonin (5-HT). Animals were treated for 4 weeks with DMI dissolved in the drinking water. Each animal drank about 20 ml water/day; the concentration of DMI in the drinking water was adjusted to provide a dose of approximately 20 mg/kg/day. Control animals drank tap water during the same period. Animals were killed 45 min after injection of reserpine. The results are expressed as per cents of the mean concentration of amine in the matched control group. The concentration of NE in the brains of control rats was 253 ± 9 ng/g, that of DA was 587 ± 23 ng/g and that of 5-HT was 196 ± 11 ng/g. Each control and experimental result represents the mean ± standard error of the mean of 8–13 observations. The asterisk (*) indicates $P < 0.05$ compared to reserpine-treated group; the mean difference between NE values from the group treated with chronic DMI plus reserpine and the group treated with only chronic DMI was significantly greater ($P < 0.02$) than the difference between the control and reserpine groups.

controls, as seen in Fig. 3. In fact, the mean difference between NE values from the group treated with chronic DMI plus reserpine and the group treated with chronic DMI alone was significantly greater ($P < 0.02$) than the difference between the control and the reserpine groups.

The brain content of DA and 5-HT was not measurably affected by the DMI alone. Long-term treatment with DMI significantly reduced retention of DA and 5-HT after reserpine injection (Fig. 3).

Age differences in the depletion action of reserpine. The same dose of reserpine (2 mg/kg) was more effective in reducing amine concentrations in the group of controls used with the acute DMI animals than in the group of controls used in the long-term DMI experiments (Figs. 1 and 3). However, the greater reserpine-induced disappearance of NE in animals treated chronically with DMI is not due to the fact that these animals were 4 weeks older than those used in the acute DMI experiments. Older rats given a single prior injection of DMI show a conservation of NE after reserpine just as reported in Fig. 1 (Table 1). In fact, in these 80-day-old rats the injection of DMI has prevented reserpine-induced depletion of NE, whereas the depletion of DA was not affected.

A comparison between "young" rats of the same age (about 50-days-old) as those used in the acute DMI experiments and rats 1 month older showed that the younger group was more sensitive than the older group to the amine-depleting effects of reserpine (Table 2). Brain NE in 50-day-old rats was reduced to 61 ± 3 per cent of the control value 45 min after a reserpine injection (2 mg/kg), whereas 80-day-old rats retained 79 ± 5 per cent of the control NE value. Brain DA was reduced to 46 ± 5 per cent of the control value in 50-day-old rats, whereas the older animals retained 78 ± 5 per cent of the control DA.

In contrast, the heart NE was reduced by reserpine to the same extent in both groups of rats. The younger animals treated with reserpine retained 49 ± 3 per cent of the control value and the older animals retained 46 ± 3 per cent of the control value.

DISCUSSION

In these experiments, several differences in the effects of acute and chronic administration of DMI emerged. As has been shown previously [22], the NE content of brain but not that of DA or 5-HT was reduced by 20 per cent after chronic DMI treatment,

Table 1. Effect of acute desmethylimipramine (DMI) on reserpine-induced release of norepinephrine (NE) and dopamine (DA) from brains of older (80-day) rats

Amine	Control	Teatment: Reserpine (2 mg/kg)	Teatment: DMI (10 mg/kg) + reserpine (2 mg/kg)
NE (ng/g tissue)	354 ± 15	282 ± 25	355 ± 17*
DA (ng/g tissue)	739 ± 41	530 ± 37	553 ± 33

* $P < 0.05$ compared to reserpine-treated group. Each value represents the mean of four to five animals.

Table 2. Effect of age on reserpine-induced depletion of heart and brain norepinephrine (NE) and brain dopamine (DA)*

Amine	Age (days)	Treatment: Control	Treatment: Reserpine (2 mg/kg)	Per cent reduction caused by reserpine
Brain NE (ng/g tissue)	50	329 ± 12	196 ± 10	61 ± 3
	80	342 ± 11	271 ± 18	79 ± 5†
Brain DA (ng/g tissue)	50	695 ± 21	316 ± 31	46 ± 5
	80	806 ± 33	634 ± 51	78 ± 5‡
Heart NE (ng/g tissue)	50	581 ± 17	283 ± 19	49 ± 3
	80	573 ± 17	264 ± 15	46 ± 3

* Each value represents the mean of five to ten animals.
† $P < 0.01$ compared to reserpine-induced reduction in 50-day-old animals.
‡ $P < 0.001$ compared to reserpine-induced reduction in 50-day-old animals.

whereas acute DMI did not affect the content of any of these amines. A single injection of DMI counteracted reserpine-induced release of NE, whereas long-term treatment with DMI did not counteract reserpine-induced release of NE and in fact caused augmented release of NE after the reserpine injection. A single injection of DMI did not significantly affect release of DA or 5-HT by reserpine, whereas chronic treatment with DMI resulted in reduced retention of these amines after reserpine. The storage mechanism for NE may be more sensitive to acute treatment with DMI than the storage mechanisms for the other amines, DA and 5-HT. Long-term treatment with DMI caused the storage mechanisms for all three amines to be more sensitive to the depleting effects of reserpine.

The finding that a single injection of DMI antagonized reserpine-induced release of NE is in agreement with previous reports of retardation of reserpine-induced depletion of NE caused by DMI [5–8], and it is in accord with additional reports which suggest that DMI has an intraneuronal site of action [9–14]. The latter reports are based mainly upon antagonism between DMI and release of NE caused by indirectly acting amines in brain and a variety of sympathetically innervated organs. In addition, Steinberg and Smith [14] found that a dose of DMI which did not block uptake of tyramine into brain slices did result in decreased conversion of tyramine to its β-hydroxylated derivative, octopamine. Decreased formation of octopamine indicated that DMI blocked the uptake of tyramine into intraneuronal vesicle containing dopamine β-hydroxylase which catalyzes the conversion of tyramine to octopamine. An action of DMI on NE storage vesicles making the contents less accessible to uptake and release processes is also consistent with the report of a slower rate of disappearance of ^{3}H-NE from brains of animals treated with one injection of DMI [17].

The accelerated release of amines by reserpine in the animals which received long-term treatment with DMI could also be the consequence of a direct effect of chronic DMI on the storage vesicles or perhaps on the firing rate of the neuronal systems involved. It is doubtful that decreased metabolism of reserpine could be responsible for the accelerated release of brain amines in animals treated chronically with DMI because release of heart NE was not accelerated in rats in which this was tested (unpublished results). However, the effect of chronic DMI on reserpine metabolism is not known. The dose of reserpine given (2 mg/kg) was selected because at short intervals after its administration to rats this dose was shown to be the ED_{50} for decrease of brain serotonin and norepinephrine [23]. Whatever the reason is, NE synthesis cannot keep up with the rate of use or cannot maintain the original pool size, since content of brain NE is always decreased after chronic DMI treatment. Although dopamine and serotonin are also more readily released by reserpine when the animal has been given long-term treatment with DMI, synthesis of these amines does keep up with the rate of use, since the total content of these amines never changes after long-term treatment with this drug.

The antagonism caused by acute DMI against reserpine-induced depletion of brain NE was not dependent upon the age of the animals. However, the experiments showed that there are significant differences between 50-day-old rats, which weight 180–200 g and are 10–20 days under the age of sexual maturity, and 80-day-old rats to the amine-depleting effects of reserpine. The younger rats were significantly more vulnerable to depletion of brain NE and DA by reserpine than were the older rats. Kulkarni and Shideman [24] have reported that infant rats (11-day-old) were much more sensitive to reserpine-induced brain catecholamine depletion than were adult rats. Subsequently it was demonstrated that the infant brains and plasma contained higher concentrations of reserpine-^{3}H than did the adult brains and plasma after injections of the same dose of the drug [25]. The capacity of the infant animal to metabolize the drug was shown to be less than that of the adult. Thus, the more rapid metabolism of reserpine in the adult animals was thought to account for at least part of the difference in sensitivity to reserpine between infant and adult animals. Similar differences in metabolism of reserpine may also exist between the two age groups of animals which we have used. However, the interaction between age and reserpine effects may be more complicated, since reserpine-induced depletion of heart NE was nearly equal in the young and old

groups of rats tested. Thus, brain amine storage sites may be less sensitive or less accessible to reserpine than are NE storage sites of the heart.

The results of these experiments agree with previous reports of an intraneuronal site of action of DMI. This action differs depending on the duration of treatment with the anti-depressant drug. It is possible that intraneuronal changes in brain and changes in the neuronal systems which utilize dopamine and serotonin as well as NE may contribute to the improvement in mental depression which occurs after long-term treatment with tricyclic anti-depressant drugs.

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