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Plasma Corticosterone and Brain Catecholamines in Stress: Effect of Psychotropic Drugs

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KEIM, K. L. AND E. B. SIGG. *Plasma corticosterone and brain catecholamines in stress: effect of psychotropic drugs.* PHARMAC. BIOCHEM. BEHAV. 6(1) 79–85, 1977. — In nonstressed rats, subcutaneous administration of haloperidol (HAL) and large doses of diazepam (DZ) increased plasma corticosterone (CS). Hypothalamic norepinephrine (NE) was lowered significantly by desmethylimipramine (DMI), HAL and, to a lesser extent, by DZ and phenobarbital (PHB). In rats pretreated with either DZ, DMI, HAL or PHB the restraint-induced rise of CS was diminished, DZ being most potent. CPZ had a variable effect, slightly increasing or decreasing the CS response. Pretreatment (16 hr) with pargyline (PA) did not affect the CS rise to stress. The reduction of hypothalamic NE evoked by restraint was attenuated by DZ, and to a lesser extent, by PHB and HAL. Restraint of PA-treated rats did not lower the PA-elevated hypothalamic NE. The stress-induced increase in hypothalamic dopamine was prevented by CPZ and, partially, by PHB. It is emphasized that the net plasma CS and brain catecholamine changes in response to stress are dependent on the drug-induced neuroendocrine feedback state prevalent immediately before commencement of the stress procedure.

Stress Corticosterone Brain catecholamines Antidepressants Neuroleptics Tranquilizers Hypothalamus

IT HAS been proposed that the resting secretion of adrenocorticotropin hormone (ACTH) is modulated by central biogenic amines influencing those hypothalamic cells which produce corticotropin releasing hormone. Catecholamines as well as indolamines have been implicated, and it is still debated whether one or more amines are involved in the basal, circadian and stress-evoked secretion of ACTH. Moreover, it remains controversial whether the amines exert an inhibitory [12,37] or facilitatory [5,27] action on basal ACTH secretion. It is also claimed that brain amines play no significant role in the control of ACTH secretion [1,8].

In contrast to the uncertainty of a biogenic amine control over resting ACTH secretion, the stress-induced release of ACTH is consistently associated with a decrease in cerebral norepinephrine (NE) and an increase in its turnover (see [37] for references). Minor tranquilizers not only prevent the stress-evoked increase in plasma corticosterone (CS) [19] but also inhibit the acceleration of NE turnover [22,28]. However, studies with other psychoactive agents known to affect aminergic mechanisms, e.g. chlorpromazine, have resulted in controversial data concerning their effects on the pituitary-adrenal axis [9].

The aim of this study was to examine the effects of representative psychoactive drugs on stress-induced changes in prosencephalic norepinephrine and dopamine, and on plasma CS as an index of ACTH secretion. In part, we also sought to further elucidate the possible physiological association between central catecholamines and ACTH secretion in continuation of our earlier work [17,33].

METHOD

Male Sprague-Dawley rats were housed individually for two weeks after they had been received at 44–55 days of age. Food and water were available ad lib, and the animals were kept in an isolated room with regulated temperature (23°C), humidity and illumination. The light period was from 0600 to 1800 hr. The body weight of the rats was approximately 295 g at the time of experimentation.

Restraint stress was effected by placing a rat in a plastic cylinder of 7 cm dia. and of variable length. Adequate ventilation was provided by means of holes in the sides and front end of the tube. The restraint duration was 30 min.

The experimental design was as follows: each experiment utilized 56 rats. Each drug was evaluated at three doses and compared to appropriate controls. A basal control group (n = 8) was included in each and was killed at 0800 hr, which was zero time for the following subsequent experimental procedures. The remaining 48 experimental rats were divided into a nonstress and a stress group. Each of these two groups was further divided into four subgroups of 6 rats each. One subgroup was injected with an appropriate placebo, and the remaining three subgroups received different doses of the test drug. Following injection of either placebo or drug, rats were returned to their home cages and left undisturbed for 1 hr. At this time, the

rats in the nonstress group were killed to determine whether the drugs, per se, altered the prestress resting hormone levels. The rats in the stress group were then restrained for 30 min and decapitated immediately thereafter.

Trunk blood was collected through siliconized funnels into tubes which contained 14 mg EDTA. Plasma was obtained by centrifugation (3000 rpm for 20 min at 4°C) and was frozen. The brain was rapidly removed from the skull and placed on ice. Two parts were dissected from the ice-cooled brains: the telencephalon, which consisted of the frontal pole anterior to the caudate-putamen complex (mean sample weight 191.2 ± 7.6 mg), and the hypothalamus which was represented by a tissue block extending from behind the chiasma to the mammillary nucleus, the lateral borders being approximately 2 mm from the midline and the dorsal border just above the arcuate nucleus (mean sample weight 102. 6 ± 9.8 mg). Following weighing, the samples were homogenized in 0.4 N perchloric acid and frozen.

Norepinephrine (NE) and dopamine (DA) eluates were prepared according to the method of Horst *et al.* [16]. Supernatants of the tissue homogenates were obtained on the day of assay, and the specific catecholamines were differentially eluted from a Dowex AG50W-X4 (Na^+ form) column. A standard curve was determined with each assay. Recovery of both catecholamines was consistently between 70 and 80%. NE and DA were determined fluorometrically according to the method of Laverty and Sharman [21].

Plasma CS was assayed according to the spectrofluorometric method of Mattingly [25] with minor modifications [17]. A CS standard curve was prepared on each assay day. Recovery was not less than 80%, and precision was greater than 95%. The in vitro reactivity of the adrenal to ACTH (Cortrosyn, Organon) was measured by assaying CS (ng CS/mg adrenal/2 hr) released from adrenal fragments as previously described [17].

The following drugs were administered subcutaneously 1 hr prior to the sacrifice of the nonstress group and the commencement of restraint of the stress group: desmethylimipramine hydrochloride (DMI), chlorpromazine hydrochloride (CPZ), haloperidol (HAL), diazepam (DZ), and phenobarbital sodium (PHB). Pargyline hydrochloride (PA) was administered intraperitoneally 16 hr before restraint. When appropriate, the drug dose was calculated in mg of its salt. DZ was used as the commercially available injectable. A 0.1% solution of HAL was prepared in a mixture of 0.1 N HCl, absolute ethanol, and water in a volumetric ratio of 1:2:100. The drugs were injected in a constant volume of 1 ml/kg body weight, except for 10 mg/kg DZ which was administered at 2 ml/kg. Saline served as the vehicle control except for the experiments with HAL and DZ in which acidified ethanol-saline and the commercial vehicle were used respectively. (Commercial vehicle: each ml contains benzyl alcohol 0.015 ml, ethyl alcohol 0.1 ml, propylene glycol 0.4 ml, sodium benzoate 48.8 mg benzoic acid 1.2 mg, water for injection qs 1 ml, pH = 6.55.)

Statistical differences in hormone or catecholamine concentration between the means of placebo-treated and drug-treated groups from nonstressed and stressed animals were determined by an unpaired Student's *t*-test. Sets of three *t*-tests (placebo vs each one of three different doses of each drug) were carried out so that the overall significance was set at 0.06. However, to provide an estimate of significance within any single *t* analysis, the probability level was adjusted to 0.02 according to Bonferroni *t* statistics for simultaneous statistical inference [26]. Thus, a difference with $p<0.02$ was regarded as significant.

RESULTS

The subcutaneous administration of various placebos (saline, acidified ethanol-saline and DZ vehicle) elicited a small stress response in rats. CS often doubled 1 hr after administration from the basal range of 4.7–5.5 μg%. Simultaneously, hypothalamic NE decreased by an average of 11% from a basal range of 1.87–2.25 μg/g. In 4 of the 5 experiments in which DA was determined (DA was not measured in the DMI experiment), vehicle administration did not significantly alter the DA content when compared to the basal range of 0.41–0.55 μg/g; in one experiment hypothalamic DA was significantly reduced. In 3 of 6 experiments telencephalic NE was unaffected by vehicle administration while it was reduced in the remaining 3 experiments. The magnitude or frequency of the observed changes did not depend upon a particular placebo.

When placebo-treated rats were restrained for 30 min, marked changes in all biochemical parameters occurred. Pooling data from the individual experiments, plasma CS increased by approximately 700%. Hypothalamic NE and telencephalic NE decreased 33%, whereas hypothalamic DA rose 28%. The difference in values between the placebo-treated, nonstressed and the placebo-treated, stressed rats represented the control response to restraint and was used in the evaluation of the psychoactive drugs. Since alterations in telencephalic NE were qualitatively similar to changes in hypothalamic NE, the telencephalic data are, though described in the text, not illustrated.

Antidepressants

DMI, in doses of 3, 10 and 30 mg/kg SC, did not raise plasma CS in nonstressed rats more than that of a saline placebo. However, the restraint stress-induced increase in CS was significantly attenuated by 10 and 30 mg/kg DMI ($p<0.01$ and $p<0.02$ respectively, each $df = 12$; Fig. 1). At all three doses, DMI significantly reduced hypothalamic NE (Fig. 2) and telencephalic NE in rats kept in their home cage for 1 hr following the injection. The stress-induced depletion of hypothalamic NE in placebo- and DMI-treated rats was the same.

A 16 hr pretreatment with PA, administered at a dose of 100 mg/kg IP, raised plasma CS 2.5 times over placebo-treated controls (Table 1). The stress-evoked CS increase in PA-pretreated rats was not different from that of vehicle-treated rats. The monoamine oxidase inhibitor increased hypothalamic NE and DA ($p<0.001$, each $df = 9$) as well as telencephalic NE in the nonstressed rat. Thirty min of restraint had no effect upon these elevated amine levels.

Neuroleptics

When compared to vehicle injection, CPZ (1, 3 and 10 mg/kg SC) did not change plasma CS significantly in the nonstressed rat. CPZ showed a trend for a dose-dependent increase in plasma CS evoked by restraint; but only the highest dose was significantly different from the vehicle-treated stress group ($p<0.02$, $df = 10$, see Fig. 1). In the nonstressed rat, hypothalamic NE and DA as well as telencephalic NE remained statistically unchanged when compared to the appropriate controls (Figs. 2 and 3). CPZ

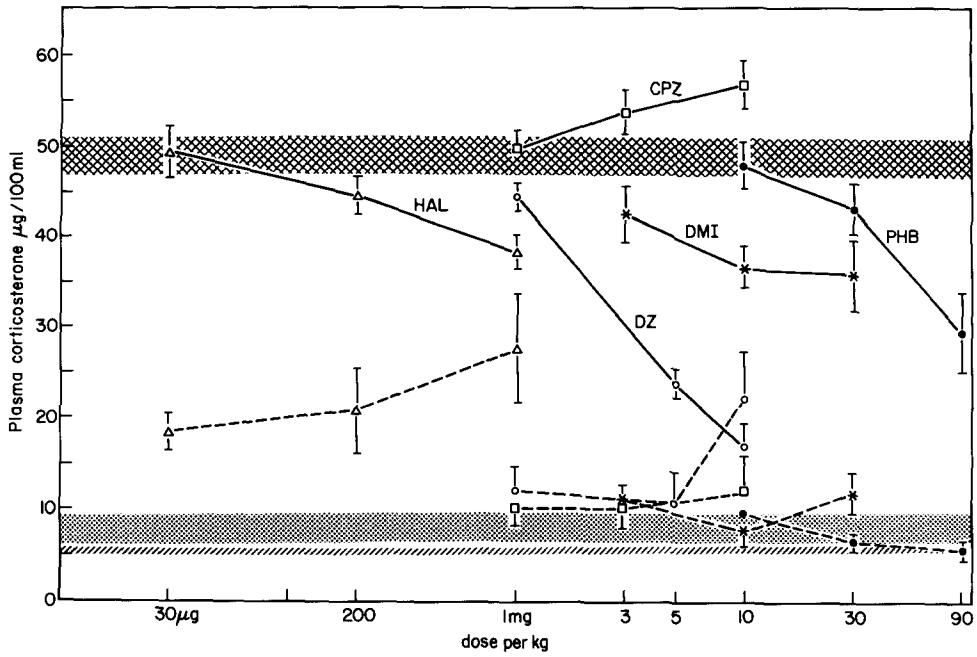

FIG. 1. Effect of psychotherapeutic drugs on basal and stress-induced plasma corticosterone in rats. Ordinate: plasma CS in μg/100 ml; Abscissa: log dose of the drugs. Narrow diagonal band (bottom): basal plasma CS ± SEM; Stippled band (middle): plasma CS ± SEM in placebo-injected, nonstressed rats; Crosshatched band (top): plasma CS ± SEM in placebo-injected rats after 30 min restraint. Solid lines connect data points representing mean plasma CS ± SEM after stressing rats pretreated with various drugs. Interrupted lines indicate the mean plasma CS ± SEM obtained when the corresponding drugs were administered to rats without subsequent exposure to restraint stress (home cage effect). The vertical bars indicate the standard error of the mean. △---△ haloperidol (HAL); □---□ chlorpromazine (CPZ); ○---○ diazepam (DZ); *---* desmethylimipramine (DMI); ●---● phenobarbital (PHB).

TABLE 1

PARGYLINE (PA) AND RESTRAINT STRESS

	Plasma CS μg%	Hypothalamic NE μg/gm	Hypothalamic DA μg/gm
basal level	5.9 ± 0.2	2.03 ± 0.09	0.48 ± 0.01
vehicle + nonstress	6.4 ± 0.5	1.96 ± 0.03	0.48 ± 0.04
PA + nonstress	15.4 ± 3.3	3.45 ± 0.15	0.89 ± 0.06
vehicle + stress	57.9 ± 4.5	1.72 ± 0.14	0.65 ± 0.04
PA + stress	56.4 ± 4.1	3.55 ± 0.04	0.83 ± 0.05

had no effect on the restraint stress-induced depletion of hypothalamic NE (Fig. 2) or telencephalic NE. Although there was a definite trend toward preventing the stress-induced increase in hypothalamic DA (Fig. 3) it was only at the lowest dose that CPZ was significantly effective ($p<0.02$, $df = 9$).

HAL caused a dose-dependent increase in resting plasma CS to a level of 27.6 μg% at the highest dose of 1 mg/kg. At this dose the stress-induced rise of plasma CS was diminished by 22%. This reduction was statistically significant ($p<0.01$, $df = 10$; Fig. 1). Hypothalamic NE was diminished by HAL in rats kept in the home cage for 1 hr reaching significance at the highest dose ($p<0.01$, $df = 10$; Fig. 2). Hypothalamic DA content was not significantly decreased by HAL (Fig. 3). The stress-induced decrease of NE tended to be diminished by HAL in doses of 0.2 and 1 mg/kg, although the values did not statistically differ from those obtained from vehicle-treated animals. Also, the stress-induced increase in DA was not significantly altered by HAL (Fig. 3).

Repeating the experiment with the neuroleptics using only the highest dose of CPZ and HAL (i.e., 10 and 1 mg/kg SC, respectively) the initial results were essentially confirmed except that the potentiating effect of CPZ on the plasma CS response to restraint did not occur.

Minor Tranquilizers

At doses of 1 and 5 mg/kg SC, DZ did not affect plasma CS significantly in animals kept in their home cages; however, a rise to 23 μg% was observed 1 hr following an injection of 10 mg/kg ($p<0.05$, $df = 7$). The restraint-induced increase in plasma CS was progressively diminished with increasing doses of DZ, significantly attenuating the CS response at doses of 5 and 10 mg/kg ($p<0.01$, $p<0.001$; each $df = 10$; Fig. 1). DZ, in all doses tested tended to reduce the NE content of the hypothalamus, but this never reached a statistical difference (Fig. 4). Following DZ, hypothalamic DA (Fig. 5) and telencephalic NE did not differ from that of controls. DZ at 5 and 10 mg/kg prevented the stress-induced reduction of the NE in the hypothalamus ($p<0.001$ and $p<0.01$, respectively; each $df = 10$; Fig. 4) and in the telencephalon ($p<0.01$, $df = 9$). On the other hand, the stress-evoked rise in hypothalamic DA was only slightly reduced (Fig. 5).

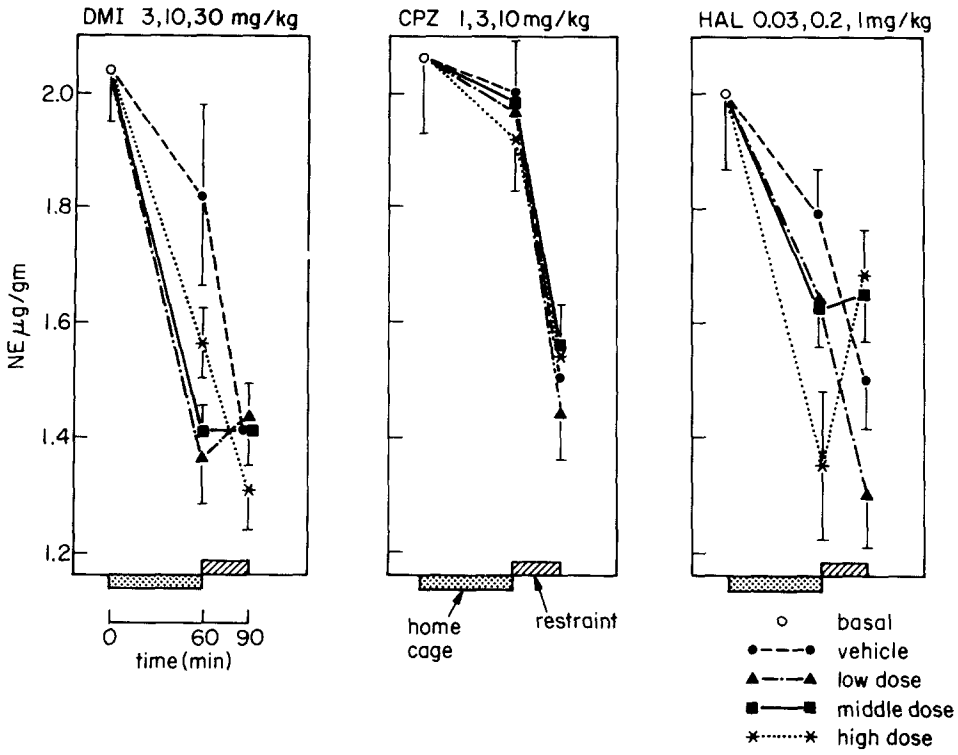

FIG. 2. Effect of psychotherapeutic drugs on hypothalamic norepinephrine.

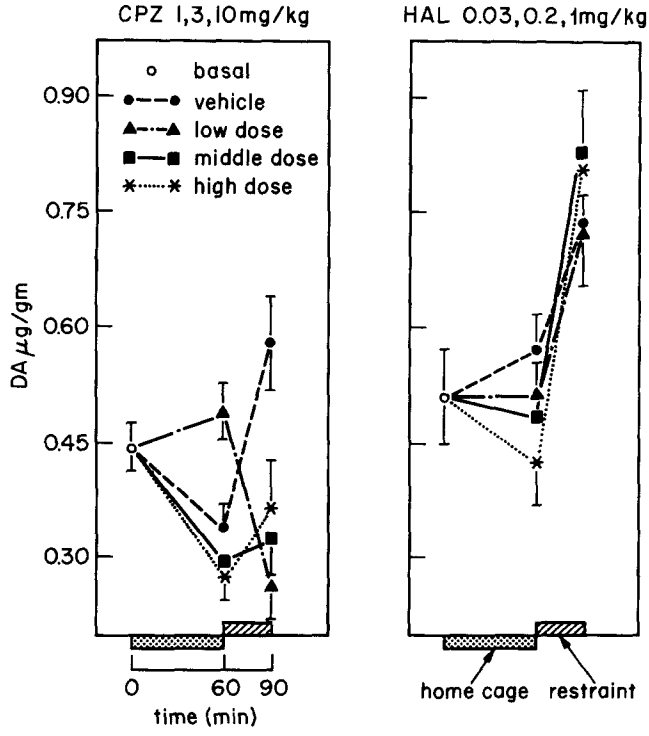

FIG. 3. Effect of chlorpromazine and haloperidol on hypothalamic dopamine.

Plasma CS of PHB-treated animals was not different from that of nonstressed, placebo-treated rats. The stress-induced CS response was attenuated in a dose-related fashion (Fig. 1), becoming highly significant at the highest dose ($p<0.001$; $df = 21$). In nonstressed rats 90 mg/kg PHB also reduced the hypothalamic NE content significantly ($p<0.01$, $df = 21$) but hypothalamic DA remained unaffected at all doses studied. The stress-induced lowering of hypothalamic NE content was attenuated only by the highest dose of PHB ($p<0.001$, $df = 21$; Fig. 4), whereas the restraint-evoked increase in hypothalamic DA was prevented by 30 and 90 mg/kg PHB (both $p<0.01$, $df = 22$; Fig. 5).

In Vitro Reactivity of the Adrenal Gland to ACTH

In order to establish that those agents which attenuated the stress-induced rise in plasma CS (i.e. DZ and PHB) did not exert their effect, at least in part, peripherally, the isolated adrenals from drug-treated, nonstressed rats were examined for their reactivity to a standard dose of ACTH.

These experiments which are summarized in Fig. 6 show that the in vitro production of CS in response to exogenously applied ACTH in DZ and PHB pretreated rats did not differ from that of rats pretreated with appropriate placebos. However, CPZ, but not HAL significantly increased the in vitro release of CS by ACTH from the adrenal gland.

DISCUSSION

The results from this study show that the administration of a placebo and of several psychotropic drugs may cause significant changes in neuroendocrine parameters. After most placebo injections, plasma CS increased [3], while hypothalamic and telencephalic NE decreased slightly with little effect on hypothalamic DA. In several instances the drugs caused additional changes in nonstressed animals kept

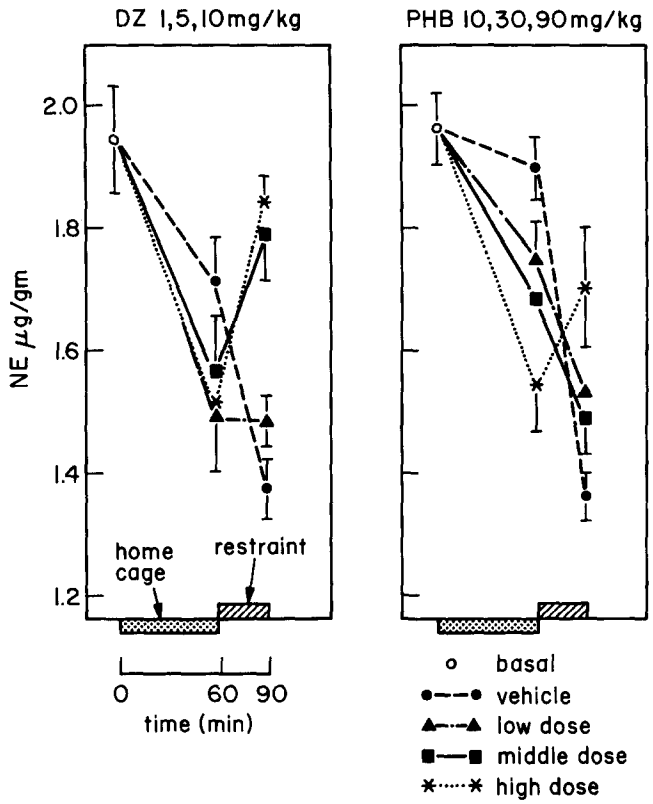

FIG. 4. Effect of minor tranquilizers on hypothalamic norepinephrine.

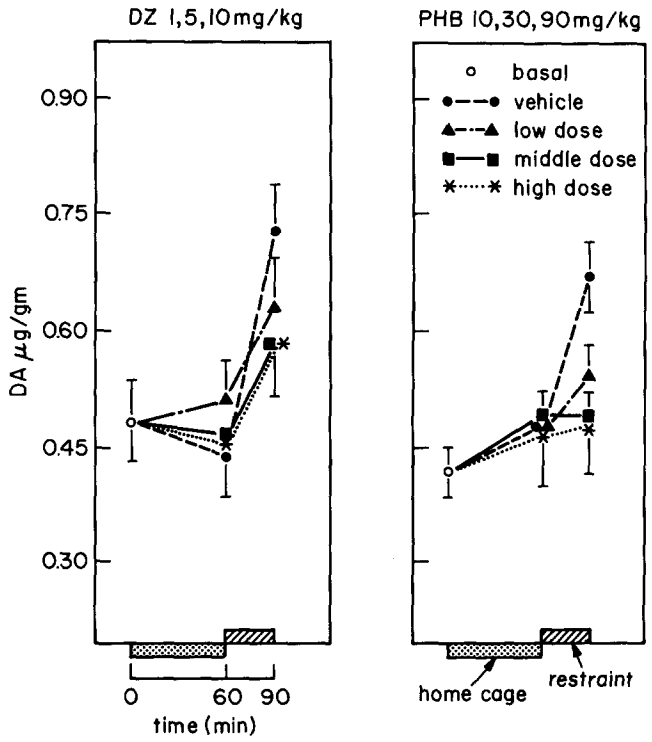

FIG. 5. Effect of diazepam and phenobarbital on hypothalamic dopamine.

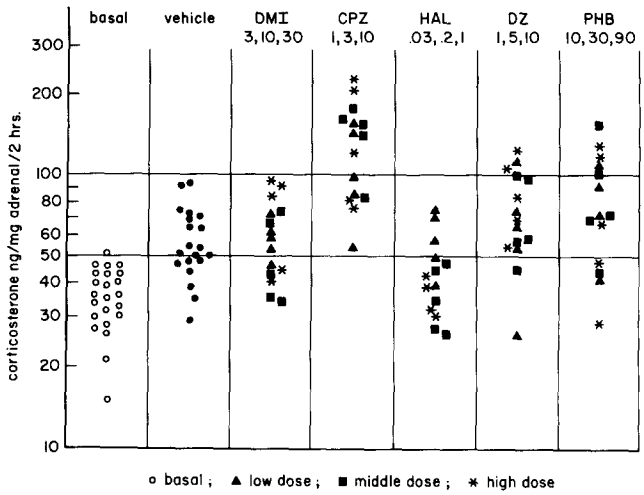

FIG. 6. In vitro adrenal reactivity to synthetic ACTH in adrenals derived from rats pretreated with centrally active drugs.

in their home cage. Thus, PA (16 hr after its administration) HAL and, in confirmation of others [24], large doses of DZ increased resting plasma CS. The observation that DMI and CPZ did not alter basal CS secretion corroborates the finding that only large doses of CPZ elicit ACTH release [20] whereas small doses are without such an effect [28].

Several of the drugs studies also altered the central monoamine content in non-stressed rats. The marked increase in amines following PA has been described before [23]. The diminution of hypothalamic NE by DMI does not corroborate results of others [31] who have measured NE 6 hr after administration vs 1 hr in our experiment. CPZ, HAL and PHB are also known to decrease brain NE [2,6]. The lack of an effect of DZ on the NE concentration in the thalamic-midbrain region 5 hr after injection [36] is not inconsistent with our finding demonstrating an insignificant decrease of hypothalamus NE 1 hr after administration.

Superimposed on the effects in nonstressed rats, the various prototypes of psychotropic drugs altered the response to stress. When a restraint stress of 30 min duration was imposed on placebo-treated rats, plasma CS and hypothalamic DA increased whereas hypothalamic and telencephalic NE decreased. These data confirm our earlier results [17] and are consistent with the finding that immobilization stress increases NE turnover and decreases DA turnover [7].

In PA-treated rats the CS response to restraint remains unchanged when compared to untreated animals. Other stressors (e.g., ether) are known to either decrease [6] or increase [1] plasma CS in animals pretreated with an MAO inhibitor. The restraint stress-induced bioamine alterations no longer occur after PA; the concentration of these amines in stressed rats remains as high as in the nonstressed rats. A possible explanation for the failure of restraint to release NE from the hypothalamus in PA-treated rats may be that some MAO-inhibitors attenuate synaptic transmission, as documented at least for peripheral ganglia [14,15]. Furthermore, ACTH release induced by electrical stimulation of the dorsal longitudinal fasciculus is blocked by MAO inhibition [13]. Also, the formation of a false transmitter which, when released by the neurogenic stressor, diminishes

the response to physiological stimuli, is another consideration [11].

The diminution of the restraint-elicited rise of CS by DMI is concordant with the finding that a variety of antidepressant drugs are effective in reducing plasma CS, elevated by exposing rats to the presence of dogs [29]. However, several antidepressant drugs failed to suppress the rise in plasma CS evoked by exposure to multiple stressors [19].

The contradictory results with CPZ betray its multiple actions. The persistent hypersecretion of ACTH induced by CPZ [35] may be followed by subsequent blockade of ACTH release in response to further stresses, possibly due to the reduction of corticotropin releasing hormone in the hypothalamus [6]. However, other investigators [30] claim that CPZ, at least in small doses, cannot reduce the effect of neurogenic stress on ACTH release. An interpretation of the central effects of CPZ on ACTH release is further complicated by the increased in vitro reactivity of the adrenal gland to ACTH by pretreatment of nonstressed rats with CPZ, indicating a peripheral adrenocortical effect. HAL consistently decreased the stress-induced CS-rise, without having any effect on the in vitro reactivity of the adrenal gland to ACTH. This is in agreement with the finding that HAL elevates steroids in nonstressed rat plasma, but decreases the steroid response to ketamine [10]. It should also be noted that HAL differs from CPZ in regard to the hypothalamic amine changes occurring during restraint stress.

The attenuation of stress-enhanced plasma CS levels by PHB and DZ must be central in origin since the in vitro reactivity of the adrenal gland from rats pretreated with these minor tranquilizers is not different from that of a placebo control. Moreover, DZ and, at the highest dose, PHB prevent the diminution of NE in the hypothalamus which occurs during restraint. This is compatible with the observation that minor tranquilizers block the stress-induced acceleration of NE-turnover in the hypothalamus [22].

In general, many psychotherapeutic drugs influence resting and/or stress-induced ACTH secretion. It must be considered that a plasma CS increase by drugs (e.g. HAL and large doses of DZ) in a nonstressed animal, may cause sufficient steroid feedback to influence subsequent ACTH release. Whether psychotropic drug effects can be explained in terms of their central action on monoamine neurons controlling ACTH release remains difficult to assess. However, it cannot be disregarded that several drugs which attenuate the restraint stress-induced reduction of hypothalamic NE simultaneously reduce the plasma CS response. This is true for DZ and, to a lesser extent, PHB and HAL, DMI being an exception. The widely different pharmacological actions of psychotherapeutic drugs on monoamine mechanisms may lead one to presume that these drugs may affect neurogenic events remote from the cellular substrates controlling ACTH.

As an alternative, if not additional explanation, it must be considered that catecholamines, secreted from the adrenal medulla during stress via activation of the sympathetic nervous system and carried to the pituitary by the general circulation, might act directly to modify ACTH release in rats [38]. Since it has been demonstrated that the psychotherapeutic drugs used in this study cause a diminution of sympathetic nerve activity in some species [18, 32, 34], it is possible that they diminish the plasma CS rise in part by inhibiting the release of catecholamines from the adrenal medulla occurring in response to restraint.

A simple classification of psychotherapeutic drugs on the basis of their prevention of stress-induced corticosterone release is difficult. It has been reported that amitriptyline, DZ and CPZ partially suppress the rise in plasma CS due to a severe, inescapable footshock [4]. Other investigators [19] have demonstrated that only the minor tranquilizers prevent the rise in plasma CS in response to a combination of stressors (e.g. novel environment, noise stress, with subsequent etherization); this preventive effect was not shared by a variety of antidepressant and neuroleptic drugs. While it is important to characterize the action of drugs on basal neuroendocrine parameters, the type, intensity and duration of the stressor are among the many critical independent variables which determine the effect of psychotherapeutic agents of the pituitary-adrenal response.

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