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Behaviorally Equivalent Stressors Differentially Modify the Monoamine Altering Property of *d*-Amphetamine[1]

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KLAUENBERG, B. J., M. S. KLEVEN AND S. B. SPARBER. *Behaviorally equivalent stressors differentially modify the monoamine altering property of d-amphetamine.* PHARMACOL BIOCHEM BEHAV **23**(3) 417–423, 1985.—We previously demonstrated that behaviorally equivalent heat and cold stressors interacted with *d*-amphetamine (AMPH) treatment to produce different effects in rats responding for food on a fixed ratio 15 (FR15) schedule of reinforcement [25]. The present study was carried out to determine if these stressors differentially affect the disposition of AMPH to brain and/or if the stressors alone or in combination with AMPH affect CNS monoamines in a dissimilar manner. Exposure to either heat or cold stressor produced equivalent elevations of [^{3}H]-AMPH in brain following 3 mg AMPH/kg but not 1 mg AMPH/kg. Neither stressor alone significantly altered any of the neurochemical parameters measured in any of the brain regions studied. In forebrain, heat and cold stressors interacted with AMPH treatment in different manners. Thus, although [^{3}H]-AMPH was equally elevated in stressed groups following the high dose, cold-induced stress was not associated with an increase in dopamine (DA) levels, which was observed in Nonstressed and Heat-Stressed subjects. Although serotonin (5-HT) levels were not changed by any manipulation, 5-hydroxyindoleacetic acid (5-HIAA) levels were lowered in Nonstressed and Cold-Stressed subjects following both doses of AMPH. This effect was not associated with heat-induced stress. The apparent attenuation of AMPH behavioral toxicity observed in Cold-Stressed and/or exacerbation in Heat-Stressed rats observed in the earlier study may involve a pharmacodynamic interaction of AMPH and stress with transmitter substances, including DA and/or 5-HT. The data support the view that the term "stress" should not be indiscriminately utilized, even when qualitatively different stressors are equated in terms of exposure time and behavioral consequences. This becomes especially obvious in studies in which drugs are introduced as independent variables.

Thermal stressors Amphetamine Serotonin Dopamine Rats

IT is an accepted generality that stress alters central nervous system neurochemistry [1, 31, 41, 42, 43, 46]. Several investigators have also compared the effects of different stressors upon brain neurochemistry following drug treatment [2, 30, 33]. We have recently discussed the problems associated with comparing the behavioral effects of different stressors when drugs known to affect behavior are administered concomitantly [24,25]. For example, the intensity of stress that the animal experiences is rarely established, prior to drug administration, for the stressors which are to be compared. We have first addressed this problem by developing a procedure wherein parameters of qualitatively different stressors (exposure to heated or chilled water) were systematically adjusted so that their effects on operant behavior were equated prior to introducing the drug. We subsequently reported that acute administration of AMPH interacted differentially with the heated and chilled water stressors. The behavioral suppressant effect of AMPH observed in Nonstressed control rats was also observed in the Heat-Stressed rats, even though their nondrug behavioral baseline rates were suppressed 50%. In contrast, the Cold-Stressed rats, also responding at 50% of baseline rates, were resistant to further suppression by AMPH.

The cause of the variable effect of the stressors as they interacted with AMPH was not determined, although several possibilities were discussed. For example, because the stressor manipulations were designed to reduce FR15 responding significantly, prior to AMPH treatment, the effect of response rate was considered. We concluded that, although cold water-induced stress appears to mitigate the behavioral toxic effects of AMPH, the lesser response to AMPH in this group may be interpreted as the "expected" effect when the lowered nondrug baseline rate is taken into account [14]. Thus, lowering response rates by exposure to a stressor as well as by schedule manipulation can antagonize the AMPH behavioral toxicity that is observed when nondrug baseline rates are left comparatively high. In other words, the response rate may be a factor modulating the degree of AMPH behavioral toxicity.

Although rate dependency may explain the apparent antagonism of AMPH behavioral toxicity following cold-induced stress, it was further shown that it was not sufficient

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to just lower response rates by a temperature/water-induced stress. Even though heat-induced stress lowered response rate to a level equivalent to that following cold-induced stress, it did not interact with AMPH in a rate dependent manner to likewise offer resistance to further suppression.

The augmented behavioral suppressant action of AMPH following heat-induced stress suggested that increased sensitivity to AMPH behavioral toxicity had somehow compromised the rate-dependent effect. Other exceptions to rate-dependent effects of amphetamines have been reported: when responding is controlled by strong external stimuli, or when responding is suppressed by immediate electric shock punishment procedures [18] or conditioned suppression by adventitious punishment under the Estes-Skinner [16] procedure [10, 22, 28] and when responding is maintained at very low rates (see [34] for review of exceptions to rate-dependency).

Another possible contributing factor discussed was the contrasting thermoregulatory behaviors the rats engaged in during the 15 min interval between exposure to the stressors and being placed in the operant chamber. Thus, rats exposed to the heated water stressor exhibited splayed-posture and relative immobility, behaviors favoring heat loss and reduced thermogenesis and antagonized by sympathetic activation. In contrast, exposure to the chilled water stressor resulted in behaviors favoring heat retention and thermogenesis. However, by the time the rats were placed within the operant chamber both groups were responding for food reinforcement at equal rates (i.e., 50% of control). Alternatively, the stressors may differentially alter disposition of AMPH in brain thereby exposing target tissue to higher concentrations, even though the groups received the same dose. Lastly, exposure to qualitatively different stressors might differentially affect one or more of the monoamines through which AMPH is believed to act. This in turn would confer a different pharmacodynamic action of the drug, producing varying behavioral effects. The present experiment was conducted to determine whether drug disposition and/or pharmacodynamic effects might be associated with the differential AMPH behavioral toxicity observed following heat- and cold-induced stress.

METHOD

Subjects

Thirty-six male Sprague-Dawley rats (Holtzman, Madison, WI) were housed individually under standard laboratory conditions (21–23°C, 40–50% relative humidity and lights on from 700–1900 hr). The rats were initially allowed ad lib access to food (Purina Rat Chow) and tap water.

Stressor Apparatus

The rats were immersed in two identical glass chromatography tanks (30×30×60 cm) filled with water to a depth of 20 cm. This depth of water allowed the rat to stand with its neck and ears submerged but able to breathe easily. The heated water tank was inserted in a plastic container in which hot water was circulated by a water bath (Model No. 3052, Lab-line Instruments, Chicago, IL) to maintain temperature within 0.1°C of 45°C. Temperature in the cold water tank was maintained to within 0.1°C of 20°C by careful addition of crushed ice.

Drugs

AMPH sulfate (Sigma Chemical Co., St. Louis, MO) was mixed with [^{3}H]-AMPH (New England Nuclear) achieving a specific activity of 10 μCi/mg. The solution was made fresh daily in 0.9% saline (1 or 3 mg/ml as base) and 1 ml/kg was injected IP. Saline vehicle served as a control injection.

Experimental Protocol

The experiment was designed to replicate as closely as possible the conditions utilized in the previous behavioral study [25], without actually running the rats in the operant test chambers. Thus, their body weights (460±26 g, mean±S.E.) were gradually reduced and maintained at 80% of free fed weight. To habituate the subjects to handling they were administered saline (IP) and a lubricated thermistor probe (Model 702, Yellow Springs Instrument Co., Yellow Springs, OH) was inserted 8 cm rectally once a day for 7 days prior to initiating the experiment.

Rats were randomly assigned to 9 experimental groups balanced for body weight. Their colonic temperatures (Tc) were measured with the lubricated thermistor probe which was inserted 8 cm rectally and attached to a digital telethermometer (Digitec 5810, HT series, United Systems Corp., Dayton, OH). The probe was secured to the tail with a 2 cm length of rubber tubing, slit lengthwise, and then wrapped with a Velcro® strip. Each rat was then placed in a polypropylene cage (25×15×12 cm) fitted with a wire cover. The Tc was recorded, to the nearest 0.1°C, immediately prior to drug injection, immediately after exposure to the stressor, and 30 min later, before sacrifice. The maximal increase (Heat-Stressed) or decrease (Cold-Stressed) in Tc, during 2 min immediately after exposure to the stressor, was recorded. All other Tc measurements were 1 min in duration.

The groups were administered either 0, 1 or 3 mg [^{3}H]-AMPH/kg immediately prior to the 4 min exposure to nonstress, heat-induced stress (45°C water) or cold-induced stress (20°C water) conditions. The Nonstress condition consisted of leaving the rat in the polypropylene cage, in which Tc had been measured, for an additional 4 min.

The rats were returned wet to their home cages for 30 min between Tc recording periods following the stressor and prior to decapitation.

Dissection

Rats were killed by decapitation 34 min after injection of [^{3}H]-AMPH, a time corresponding to the mid-point of the behavioral session in the previous experiments. AMPH has been shown to equilibrate rapidly following doses of 1–4 mg/kg, reaching constant brain tissue/plasma ratios within 30 min following IP injection [29,33]. Brains were removed rapidly and cut sagitally, over ice, and each half further dissected into hypothalamus, cerebellum, brain stem (to the superior colliculus) and forebrain. Samples were frozen on dry ice and stored at −70°C. Left and right brain samples were randomly distributed to groups to be assayed for AMPH or monoamines.

AMPH Extraction and Assay

Brain levels of [^{3}H]-AMPH were measured by a variation [39] of the methods of Axelrod [5] and Maickel *et al.* [29]. Unless stated otherwise, all procedures were carried out at room temperature under a hood. Tissue samples were

homogenized using a Tekmar Ultra-Turrax Tissumizer (Model SDT, Cincinnati, OH), over ice, in 0.01 N HCl (5 ml final volume) and added to 6 ml benzene and 1 ml 2.0 N NaOH. The samples were shaken in glass stoppered reaction vessels for 15 min and centrifuged for 20 min (590×g). A 5 ml aliquot of the benzene fraction was added to 2 ml 0.5 N NaOH, shaken for 10 min and then recentrifuged. A 4.5 ml aliquot of the benzene fraction was added to 1.0 ml of 1.0 N formic acid, shaken for 10 min and centrifuged as before. A 0.4 ml aliquot of the aqueous phase was added to 3.6 ml Aquasol-2 liquid scintillation cocktail (New England Nuclear, Boston, MA) and counted in a Beckman LS-150 Liquid Scintillation Spectrometer. Counting efficiency was determined by the external standard ratio procedure and CPM were converted to DPM. No correction for recovery was made since all samples were extracted at the same time. The data were converted to μg AMPH/g tissue, based upon the specific activity of 10 μCi/mg AMPH.

Monoamine and Metabolite Extraction

Monoamine extraction was performed by a modification [40] of the method of Shore and Olin [36]. Two volumes of 0.01 N HCl (2.0 nM Na_2EDTA) were added to each brain sample, followed by 33 volumes of n-butanol. The acidified butanol-tissue mixture was homogenized (Tekmar Tissumizer) over ice and 0.2 g NaCl was added to 1.2 ml aliquots.

Following 1 min centrifugation (1050×g) a 1.0 ml aliquot of the organic layer was removed and then vortexed with 1.5 ml n-heptane and 0.25 ml 0.01 N HCl for 30 sec and then centrifuged for 1 min. A 0.23 ml aliquot of the aqueous layer was added to 2.0 ml HPLC mobile phase (0.005 N $HClO_4$, 0.1 mM Na_2EDTA, 0.25 mM octane sulfonic acid-sodium salt and 10% CH_3OH (v/v)) and 20 μl ascorbate solution (28.4 μM ascorbic acid). This acid extract (containing norepinephrine (NE), DA and 5-HT) was stored at 4°C, in the dark, overnight. The remaining organic layer was vortexed with 0.23 ml of 0.1 M potassium phosphate (monobasic-dibasic, pH 7.4) and then centrifuged (1 min). A 0.23 ml aliquot of aqueous layer was added to 2.0 ml of HPLC mobile phase and acidified by addition of 5 μl 6 N $HClO_4$ and ascorbate solution (15 μl). This acidified extract (containing DOPAC, 5-HIAA and homovanillic acid (HVA)) was stored at 4°C in the dark until assayed (within 12 hr). Standards of monamines and metabolites were subjected to the same extraction procedures daily to allow correction for daily variability in recoveries and the generation of standard curves.

Due to equipment malfunction, the first series of monoamine determinations (N=1 per group) were not included in the data analysis. Final group size was three per group.

HPLC

Two hundred μl samples were injected onto an HPLC system which consisted of a Beckman Model 110A pump containing a Model 210 valve, and an Ultrasphere-ODS column (C_{18} 5 μm, 25 cm × 4.6 mm ID) maintained at 25°C by a Bioanalytical Systems (BAS, West Lafayette, IN) temperature controller. A precolumn (40×4.6 mm ID) was placed in front of the analytic column to prevent contamination of the analytical column and was repacked frequently with Spherisorb ODS particles (Applied Science, Deerfield, IL). The electrochemical detector consisted of a BAS glassy carbon electrode (Model TL 8A; +0.8 V versus auxillary electrode) and a custom built electrochemical controller unit. Peak heights and retention times were determined using a Hewlett Packard Model 3390A recording integrator. Authentic NE, DA, 5-HT, DOPAC, 5-HIAA and HVA were measured daily for linear regression quantification of experimental peaks. Retention times were stable through each series of injections.

TABLE 1

MEAN (±S.E.M.) COLONIC TEMPERATURE (°C) IN RATS EXPOSED TO HEATED OR CHILLED WATER STRESS WITH AND WITHOUT AMPHETAMINE TREATMENT

Treatment	Basal†	Stress + 2 min‡	Stress + 30 min¶
Nonstress			
Saline	37.0 ± 0.4	37.6 ± 0.3*	38.2 ± 0.2*
1.0 mg/kg	37.0 ± 0.4	37.6 ± 0.5*	38.0 ± 0.5*
3.0 mg/kg	36.9 ± 0.3	37.7 ± 0.2*	37.2 ± 0.4§
Heat-Stress			
Saline	37.2 ± 0.4	41.7 ± 0.2*	36.5 ± 0.2*
1.0 mg/kg	37.3 ± 0.3	41.5 ± 0.2*	36.1 ± 0.2*
3.0 mg/kg	37.9 ± 0.3	42.1 ± 0.1*	33.4 ± 0.2*§
Cold-Stress			
Saline	37.0 ± 0.4	32.8 ± 0.6*	34.6 ± 0.7*
1.0 mg/kg	36.9 ± 0.4	32.1 ± 0.1*	33.5 ± 0.3*
3.0 mg/kg	37.1 ± 0.5	32.3 ± 0.4*	31.3 ± 0.7*§

N=4 for all groups.
*$p<0.05$ vs. Basal (Duncan's Multiple Range Test).
†Immediately before stress.
‡2.0 min after 4.0 min stress.
§$p<0.05$ vs. Saline (Dunnett's Test).
¶Immediately before decapitation.

Experimental Design and Data Analyses

The interaction of 0, 1 and 3 mg AMPH/kg with three stress factors (Nonstress, Cold-Stress and Heat-Stress) was examined. The effects of dose of AMPH and stress upon AMPH distribution to various brain regions was partitioned by a three-way analysis of variance with repeated measures on the brain regions factor. The effects of stress, dose of AMPH and the interaction of stress and dose upon Tc and levels of monoamines or metabolites were partitioned by ANOVA with repeated measures on the time of measurement factor [47]. Individual comparisons were made by either Fisher's least-significant difference (lsd) method, Duncan's multiple range tests or Dunnett's test when appropriate. Significance level was set at 0.05 for all tests.

RESULTS

Effects of AMPH and Stress on Tc

The effects of stress and drug treatment upon Tc in the present study replicate those of our earlier report [25]. Significant main effects of stress, $F(2,27)=127.14$, $p<0.0001$, and dose, $F(2,27)=3.88$, $p<0.03$, upon Tc were obtained. Although there was no significant stress by dose interaction, the stress by dose by time interaction was significant, $F(8,54)=4.44$, $p<0.0004$. Table 1 shows that handling alone

TABLE 2

AMPHETAMINE CONCENTRATIONS IN FOUR BRAIN REGIONS OF RATS THIRTY-FOUR MINUTES AFTER INJECTIONS OF 1 OR 3 mg AMPHETAMINE/kg IP AND EXPOSURE TO NONSTRESS, 4 MIN × 20°C (COLD-STRESS) OR 4 MIN × 45°C (HEAT-STRESS)

Amph Dose (mg/kg)		Hypothalamus	Cerebellum	Brain Stem	Forebrain	Whole Brain§
1.0	Nonstress	0.59 ± 0.13‡	0.55 ± 0.05	0.61 ± 0.06	0.99 ± 0.09	0.85 ± 0.08
	Heat-Stress	0.65 ± 0.10	0.66 ± 0.10	0.72 ± 0.05	1.12 ± 0.07	0.96 ± 0.06
	Cold-Stress	0.59 ± 0.16	0.56 ± 0.06	0.62 ± 0.08	0.87 ± 0.12	0.76 ± 0.10
3.0	Nonstress	1.71 ± 0.22	1.77 ± 0.29	1.76 ± 0.29	2.64 ± 0.42	2.31 ± 0.36
	Heat-Stress	1.68 ± 0.28	2.15 ± 0.33	2.19 ± 0.26	3.64 ± 0.26*†	3.10 ± 0.25*
	Cold-Stress	2.04 ± 0.15	2.22 ± 0.21	2.27 ± 0.15	3.66 ± 0.27*†	3.16 ± 0.23*

N=4 for all groups.
*Significantly elevated compared to 3 mg/kg Nonstress, $p<0.05$ Duncan's Multiple Range Test.
†Significantly elevated compared to hypothalamus, cerebellum or brain stem, $p<0.05$ Individual Comparisons.
‡μg AMPH/g wet weight (M ± SEM).
§Whole brain values represent the sum of individual regions.

(inserting the thermistor and/or injecting drug or vehicle) in Nonstressed animals resulted in an elevation of Tc during the 34 min period. This slight hyperthermia was blocked by 3 mg AMPH/kg when measured 34 min after injection.

As in the earlier report, AMPH did not significantly influence the changes in Tc that were observed immediately after exposure to the stressor. Thirty minutes later the 3 mg/kg dose of AMPH induced a significant decrease in Tc. While 1 mg AMPH/kg also produced a significant decrease in Tc 45 min after exposure to the stressors in the earlier report, this effect was not observed in the present study 30 min after exposure to stressor.

Distribution of [^{3}H]-AMPH in Brain

Detectable quantities of [^{3}H]-AMPH were observed in all brain regions following injection of 1 mg AMPH/kg. As expected, concentrations of unchanged drug were proportionately higher after 3 mg AMPH/kg compared with the lower dose. A three-way analysis of variance with repeated measures on the brain regions factor indicated significant main effects of dose, $F(1,18)=129.97$, $p<0.0001$, and region, $F(3,54)=79.04$, $p<0.0001$. The dose by region interaction was also significant, $F(3,54)=25.57$, $p<0.0001$. Duncan's Multiple Range Tests revealed significant dose related increases in AMPH concentration in each brain region following each stress treatment. Inspection of the profiles corresponding to this interaction indicated that, with the increase in AMPH dose from 1 to 3 mg/kg, there was a selectively greater deposition of AMPH in the forebrain. Tests on the AMPH concentrations between brain regions following the low dose failed to reach significance. However, the forebrain AMPH concentration was significantly elevated compared to hypothalamus, $F(3,54)=5.27$, $p<0.05$, brain stem, $F(3,54)=3.58$, $p<0.05$, and cerebellum, $F(3,54)=3.57$, $p<0.05$, following the 3 mg AMPH/kg dose. Separate analyses of the effect of dose and stress in the forebrain indicated that neither stressor significantly altered AMPH concentrations after the low dose. Both stressors significantly elevated AMPH concentrations, to an equivalent extent in forebrain, after the higher dose (Table 2).

Effects of Stress and/or AMPH on Monoamines and Their Metabolites

The consequences of combining AMPH and stress upon brain monoamines and their metabolites were varied and complex. Generally, the most consistent changes occurred in forebrain and indicate that heat- and cold-induced stress interacted with drug treatment in a different manner. The stressors themselves did not detectably alter the various neurochemical parameters studied in any of the brain regions. Although both stressors caused a significant elevation in forebrain concentration of AMPH after the high dose (vide supra), examination of the data in Fig. 1 indicates that steady state levels of DA and perhaps the 5-HT metabolite 5-HIAA in this brain region were differentially affected by the drug-stress combination. Significant overall effects of AMPH treatment on DA, $F(2,18)=6.33$, $p<0.01$, and 5-HIAA, $F(2,18)=5.10$, $p<0.02$, levels in forebrain were obtained. Treatment with 1 mg AMPH/kg had no effect on DA levels in any of the groups. However, consistent with the behavioral data [25], when compared to the Nonstressed-0 mg AMPH/kg control group, 3 mg AMPH/kg elevated the concentration of DA significantly in the Nonstressed and Heat-Stressed groups, but not in the Cold-Stressed group. When comparisons were made with the corresponding stress-0 mg AMPH/kg group, again only the Nonstressed and Heat-Stressed 3 mg AMPH/kg groups showed significantly different levels of DA. Thus, even though AMPH concentration was elevated in both Heat- and Cold-Stressed subjects, following the 3 mg AMPH/kg dose, cold-induced stress was not associated with the increase in DA levels that was observed in the other subjects. When compared to the corresponding stress-0 mg AMPH/kg group both doses of AMPH lowered 5-HIAA levels in the Nonstressed groups. Similar comparisons failed to show any differences in the stressed groups following either dose of AMPH. However, when compared to the Nonstressed-0 mg AMPH/kg controls, drug treatment resulted in lowered 5-HIAA levels in Nonstressed and Cold-Stressed subjects given either dose of AMPH, while 5-HIAA levels were not significantly affected in any group exposed to the heat stressor.

DISCUSSION

This report presents neurochemical data which, like an earlier behaviorally oriented report [25], indicates that even when equated behaviorally prior to drug treatment, qualitatively different stressors interact with AMPH in a dissimilar manner. We previously reported that AMPH-induced suppression of FR15 operant responding was attenuated in rats that had been exposed for 4 min to water chilled to 20°C, possibly because response rates were reduced significantly before drug treatment. Exposure to water heated to 45°C had no apparent antagonistic effect on the behavioral suppressant action of AMPH. Thus, even though both stressors suppressed FR15 food reinforced behavior to an equivalent extent without drug, they interacted with AMPH to produce different effects on behavior. While these data indicated it was not sufficient to lower response rates to demonstrate rate-dependency [14] it also suggested that the means utilized to lower response rates may act in concert with AMPH to produce a greater or lesser effect than expected. We now report that these divergent observations were not a result of differences in brain AMPH disposition between the two stress groups but rather appear to involve pharmacodynamic interactions of AMPH and the stressors, expressed as changes in central nervous system monoaminergic steady-state concentrations.

The concentrations of AMPH in brain obtained here agree well with those previously reported by this laboratory [37] and others [29]. Our observation that the greatest AMPH disposition occurred in the forebrain, in both groups of stressed rats following the higher dose of drug, is consistent with an earlier report by Eison and colleagues [15]. They also found that AMPH concentrations were greatest in rostral brain regions and that these differences were magnified in the frontal cortex and hippocampus by increasing the dose of AMPH from 1.5 to 5.0 mg/kg. We have previously reported that footshock can alter the disposition of AMPH after acute or chronic administration [39]. In the present study, merely increasing the dose of AMPH from 1.0 to 3.0 mg/kg was not sufficient to magnify regional differences in Nonstressed animals. However, exposure to heated or chilled water stressors acted selectively to further increase AMPH disposition in forebrain following the high dose.

This suggests that AMPH and the stressors evaluated in this study may be interacting in a synergistic manner to affect AMPH disposition. Consistent with this suggestion is the observation that AMPH produces effects which in many respects mimic other stressors, indicating that it may act as a stressor itself [7,31]. In fact, noting that AMPH administration and exposure to stressors produce similar behavioral and neurochemical profiles, Kokkinidis and MacNeil [26] suggested that AMPH and stress may act synergistically on behavior. They reported that while exposure to isolation-induced stress or to inescapable shock had no effect on startle activity in mice, both types of stressor potentiated the effects of AMPH on startle arousal. Antelman *et al.* [3,4] and Hellman, Crider and Solomon [19] have also suggested that a link exists between the effects of AMPH and stressful stimulation and that they produce similar behavioral and neurochemical effects.

In contrast to the above cited studies, previous studies in this laboratory suggested that all stressors may not interact with AMPH to produce similar behavioral effects. For example, it was demonstrated that three qualitatively different stressors interacted with chronic AMPH treatment differentially [12]. While cold environment (7°C × 30 min) and footshock (0.5 sec × 2 mA/min × 30 min), caused little or no behavioral suppression alone, footshock interacted with chronic (6 day) administration of 2.5 mg AMPH/kg to suppress FR15 operant responding by 79%. Cold environment exposure failed to suppress behavior significantly below control levels following chronic drug treatment. Exposure to a behaviorally active stressor (and thus more intense?), cold water immersion (15°C/2 min), suppressed behavior by 47%. Unlike the footshock stressor, cold water-induced stress did not interact with chronic AMPH to further suppress operant responding. Similarly, we recently showed that AMPH *did not* interact with qualitatively different stressors to produce similar behavioral effects, even though the stressors alone had equivalent effects on behavior [25]. In the present study, the monoamine data further indicate that these qualitatively different stressors which produce equal FR15 behavioral consequences interact in a dissimilar manner with AMPH. In particular, under the conditions utilized herein, an elevation in DA levels observed in the Nonstressed and Heat-Stressed groups administered 3 mg AMPH/kg was not observed in the Cold-Stressed rats similarly treated with AMPH.

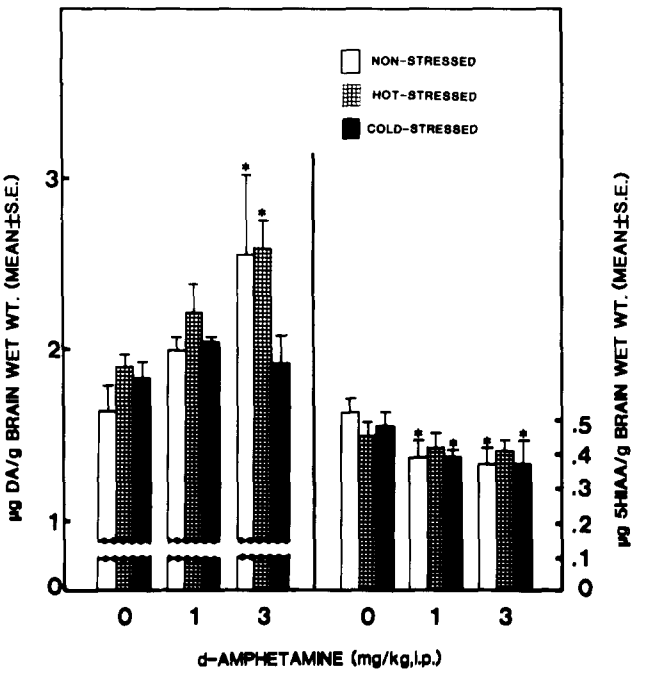

FIG. 1. Amount of dopamine (DA) and 5-hydroxyindoleacetic acid (5-HIAA) in forebrains of rats 34 min following the stress-drug treatment. N=3 for all groups. *Indicates a significant difference from the Nonstressed 0 mg amphetamine/kg control group at the 0.05 level (Fisher's least significant difference test).

Early reports indicated that acute exposure to a stressor lowered brain levels of DA by increasing catabolism [8]. This is usually seen following severe stress, which also increases NE turnover [27]. Thierry *et al.* [44] reported decreased DA levels in the nucleus accumbens and frontal cortex following a mild footshock-induced stress in rats pretreated with the catecholamine synthesis blocker, α-methyl-*para*-tyrosine, indicating enhanced utilization. This mild stressor did not alter NE. Increases in frontal cortex DA synthesis and metabolism have also been reported in the face of an increase in

DA steady state levels following mild footshock-induced stress [32]. Such observations suggest that DA levels and utilization may be more sensitive than NE to the effects of stressors. It is therefore not surprising that we observed a change in steady-state level of DA under conditions in which NE levels were unaffected.

In an early study, which evaluated the effect of various stressors and AMPH on rat brain NE and DA, Moore and Lariviere [30] failed to find significant differences between levels of these catecholamines in brains of rats injected with 3 mg AMPH/kg and swum at 23° or 37°C for 4 hr. Since the rats were described as exhausted, the procedure was probably severe enough to have produced maximal effects on catecholamines in both groups. Unfortunately, as discussed above, these studies did not adquately control for differential intensity of stress induced by qualitatively different stressors. Had the two thermal stressors in the Moore and Lariviere study been adjusted in order to produce equivalent and milder magnitudes of stress, a differential response of catecholamines to the stressors plus AMPH may have emerged. The monoamine data in the present report and in those cited above support such a prediction.

The fact that the Heat-Stressed and Nonstressed groups show elevated DA, following the high dose of AMPH, suggests that the DA differences may be a consequence of the (behavioral?) effects of stress rather than a cause or mediator. The increase in activity (shivering, thermogenic movement) in the AMPH-Cold-Stressed groups as compared to the control group is consistent with the observation that DA receptor stimulation increases activity whereas DA receptor blockade decreases motor activity [13, 17, 20]. Further, since the level of motor activity has been shown to affect brain catecholamine levels [38], it is possible that the higher level of motor activity involved in thermogenesis produced greater catabolism of DA in the Cold-Stressed group. Alternatively, the fact that AMPH suppressed behavior in a dose-dependent manner in control and Heat-Stressed rats and not in Cold-Stressed rats [25] suggests the common denominator has something to do with the drugs effect on DA neurotransmission. Either the cold stress attenuated such an effect or the heat stress interacted with AMPH to sensitize the rats to further behavioral suppression through enhanced dopaminergic neurotransmission. Whatever the underlying mechanism is, it is important to note that only after combined AMPH-stressor manipulation did differences in DA appear between the two stress groups.

Numerous reports have shown decreases in NE levels proportional to the severity of the stress [2]. No alterations in NE levels were observed in the present study, suggesting that the stressors were relatively mild *(vide supra)*. It is possible that a stress-induced increase in utilization of NE is compensated for by a corresponding increase in synthesis of NE, similar to that reported by Thierry and associates [21,45] and others [11]. Since we did not measure MHPG in brain, we cannot determine if such as the case.

The observed changes in 5-HIAA are difficult to interpret. Although heat-stressed animals were not different from control animals on this measure, one may speculate that heat-induced stress interacts with AMPH to *increase* 5-HIAA levels that are decreased by AMPH alone. This interpretation is consistent with a previous report that showed no changes in 5HT but increased levels of 5-HIAA in proportion to the severity of the footshock stressor [9]. Since heat-induced stress, but not cold-induced stress, interacted with AMPH to block an AMPH-induced decrease in 5-HIAA, heat-induced stress may interact with AMPH to produce a more severe stress response. As we noted above, the important observation is that differences in response to otherwise equivalent stressors emerged from their interaction with AMPH.

Since all stressors have some specific (unique) effects, they can be expected to elicit differing stress responses. Selye notes that "even qualitatively different stimuli of equal toxicity (or stressor potency) do not necessarily elicit the same syndrome in different people" and that "qualitatively distinct stimuli differ only in their specific actions" ([35], p. 33).

This report emphasizes our contention that the generality of drug-stressor interactions is limited by the nature of the stressors. Examination of data from an earlier report [6] reveals results consistent with this contention. In that study, the simultaneous influence of swim and cold- or heat-induced thermal stress on adrenal catecholamines was examined in rats exposed to water temperatures ranging from 4°C to 47°C. Although exposure to either 4°C or 47°C resulted in equivalent survival times, the cold stressor produced marked catecholamine depletion while the heat stress elevated catecholamines. Thus, even when careful measures are taken to compare behaviorally equivalent stressors, unitary or general neurochemical indices are not readily apparent. The effect of stressors on any index must be considered within the context of the accompanying conditioning factors and specific stressor responses.

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