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BRIEF COMMUNICATION

Cold-Restraint Stress and Urinary Endogenous β-Phenylethylamine Excretion in Rats[1]

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SNODDY, A. M., D. HECKATHORN AND R. E. TESSEL. *Cold-restraint stress and urinary endogenous β-phenylethylamine excretion in rats.* PHARMACOL BIOCHEM BEHAV **22**(3)497–500, 1985.—Stress applied to humans increases the urinary excretion of the endogenous amphetamine-like substance β-phenylethylamine (PEA), a potentially common mediator of amphetamine and stress effects. The present study was conducted to determine if cold-restraint stress in the rat could represent an animal model for stress-induced changes in PEA disposition in humans. The stressor markedly elevated the urinary excretion of endogenous PEA in a manner that was not attributable to changes in urinary pH, glomerular filtration rate or in food consumption. In addition, a large diurnal variation in PEA excretion was noted. The data suggest that the variables responsible for stress-induced alterations in endogenous PEA disposition in humans and rats are generally similar. However, they also indicate that in rats, in contrast to humans, PEA disposition is subject to diurnal changes.

Phenethylamine Stress Rats Excretion

β-PHENYLETHYLAMINE (PEA), a structural analog of amphetamine (α-methyl PEA), is a naturally-occurring constituent of blood, brain, urine and a variety of peripheral tissues including adrenal medulla [2, 9, 12, 15, 16, 20] that has been indirectly implicated in several physiological or pathophysiological processes. For example, PEA has the properties of an indirectly-acting sympathomimetic in the autonomic nervous system [10,14] and therefore has the capability of being involved in the regulation of blood pressure. In addition, PEA is a stimulant of motor activity in rodents [4, 8, 11], a reinforcer of drug-taking behavior in animal models of human drug abuse [6, 19, 22] and a releaser of catecholamines within the central nervous system [13,17], suggesting that it is also capable of functioning as an endogenous euphoriant as well as mediating natural reinforcement processes. Finally, the urinary excretion of endogenous PEA is significantly greater in paranoid schizophrenic patients than in other schizophrenics or normals [18]. Since high doses of amphetamine elicit a syndrome in non-schizophrenic humans that is clinically quite similar to paranoid schizophrenia [3], the former finding also implicates endogenous PEA as an etiological factor in the genesis of this psychiatric disorder. Unfortunately, these observations cannot be construed as demonstrating the existence of a causal relationship between changes in the disposition of endogenous PEA and alterations in function since they only provide indirect evidence for such a relationship.

Direct evidence concerning the importance of PEA could begin to be provided if it could be shown that some factor which has been implicated in the etiology of hypertension, psychiatric illnesses and reinforcement processes also affected the disposition of PEA. Recent data suggests that environmental stress may be such a factor. Thus numerous observations indicate that the cardiovascular, neurochemical, behavioral and psychiatric consequences of environmental stress and of amphetamine administration are quite similar (see [1] for a review), and Paulos and Tessel [12] have demonstrated that stress in normal humans markedly elevates the urinary excretion of endogenous PEA. Since these increases in PEA excretion were not attributable to changes in diet, urine pH or glomerular filtration rate, it seems likely that stress increased the amount of PEA available for excretion. However, further tests of the hypothesis that stress-induced changes in PEA disposition may mediate some of the sequelae of stress requires the availability of a suitable animal model. The purpose of the present paper was to determine whether the stressed rat could represent such a model.

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TABLE 1

THE EFFECT OF STRESS AND DIURNAL RHYTHM ON ENDOGENOUS URINARY β-PHENETHYLAMINE (PEA) EXCRETION, OTHER URINARY PARAMETERS AND FOOD INTAKE

Time Period	Condition	PEA Excretion (ng)	Urine Volume (ml)	Creatinine Excretion (mg)	Urine pH	Food Consumption (g)
Day	Non-stress	771 ± 77	4.8 ± 0.7	1.2 ± 0.2	6.9 ± 0.1	4.7 ± 0.7
	Stress	1236 ± 157‡	4.6 ± 0.5	1.4 ± 0.2	6.9 ± 0.2	4.2 ± 0.7
Night*	Non-stress	1522 ± 193	9.0 ± 1.1	3.3 ± 0.5	7.2 ± 0.1	16.4 ± 1.2
	Stress	1816 ± 123†	9.3 ± 0.8	3.0 ± 0.2	7.2 ± 0.1	15.9 ± 1.0

Each value represents the mean ± 1 S.E.M. of 10 observations.
*All Night values are significantly increased ($p<0.01$) compared to Day values except urine pH.
†$p<0.05$; ‡$p<0.01$; compared to non-stress condition during the same time period.

METHOD

Animals

Male sprague-Dawley rats weighing 160–180 g (Sasco, Omaha, NE) served as the experimental subjects and were individually housed under a 12 hr light-dark cycle throughout the study. Rodent chow and water were available ad lib.

Stress Application and Urine Collection

The 48 hr experiment was initiated by placing a subject within an aluminum metabolic chamber (Acme Metal Products, Chicago, IL) at 0730 hr at ambient temperature. Exactly 24 hr later, the rat was briefly removed from the chamber, placed into a plastic rat restrainer (Harvard Apparatus Co., Inc., South Natick, MA) and returned to the chamber. The chamber was then immediately put into a cold-room (4°C) for a two-hour period. After this, the chamber containing the freely-moving animal was again placed in the original ambient-temperature environment, and allowed to remain there for the succeeding 22 hr period. Food and water were available ad lib except that the rodent chow was powdered to prevent the animals from transporting the food into the chamber where it might drop into and contaminate the urine collection.

Urine was collected by placing a vial immediately beneath the collection funnel of the chamber. The vial was kept cold during collection by surrounding it with ice and a styrofoam lid, through which the vial protruded, was used to reduce the rate of melting and to prevent the chamber *per se* from cooling. Vials were changed and fresh ice added at 12 hr intervals. Otherwise the animals were left undisturbed. A hardware-cloth screen was placed at a 45° angle within the collection funnel to prevent contamination of the collected urine by feces. After removal of a vial from the ice, the vials were placed in a −70°C freezer until assay.

Assay Procedures

Urinary PEA content was determined by the method of Paulos and Tessel [12]. Thawed urine (1 ml) was alkalinized using 0.5 ml of 0.5 M phosphate buffer. An internal standard (40 ng of deuterated PEA; Merck, St. Louis, MO) was then added to the alkalinized urine and the PEA extracted into diethylether with mixing. After centrifugation of the aqueous-ether solution, the ether supernatant was removed and the PEA contained in this fraction was back-extracted into 300 μl of 0.1 N HCl. The ether layer was subsequently removed by aspiration and the water contained in the remaining acid layer evaporated at 65–70°C under a stream of argon. The PEA in the residue was then derivatized using 5% pentafluropropionyl imidazole (PFPI; Pierce Chemical Co., Rockville, IL) in 200 μl of HPLC-grade ethylace acetate by heating the tubes at 80°C for 12 min. Following this, the sample was dried to a residue by heating the tubes at 65–70°C under argon. The derivatized PEA was reconstituted in 50 μl of ethyl acetate for injection into a Ribermag R10-10 quadruple gas chromatograph-mass spectrometer (GCMS). Gas chromatography was carrier out on a 30 M×0.32 mm (i.d.) fused-silica capillary column, and the GCMS was set to focus on ions of 104 and 107 amu to detect PEA and [$^2H_4$]PEA, respectively. Creatinine was measured colorimetrically as described in [5].

Statistical Analysis

Results are expressed as a mean±S.E.M. Analysis of variance and Student's *t*-test for paired observations were used to determine statistical significance.

RESULTS

Urine values obtained during the two 24-hr periods as well as the two 12-hr periods preceding and the two following the initiation of cold restraint stress, are presented in Table 1. The amount of PEA excreted during the pre-stress 24 hr control period was 2.3±0.2 μg. Stress increased the amount of PEA excreted, $F(1,36)=7.02$, $p<0.025$, and subsequent statistical analysis indicated that the stress-induced increase occurred during both the day ($p<0.01$) and the night ($p<0.05$). In contrast stress did not alter urine volume, pH or creatinine content, or the amount of food consumed. No significant interactions between the application of stress and diurnal variation were obtained with any dependent measure.

Significant nocturnal increases were evident in all measures except urine pH; amounts of urinary PEA, $F(1,36)=21.6$, $p<0.01$, and creatinine, $F(1,36)=46.2$, $p<0.01$; urine volume $F(1,36)=42.1$, $p<0.01$, and the amount of food consumed, $F(1,36)=162$, $p<0.01$. A statistically significant correlation between the amounts of PEA and creatinine excreted during the day under the stressed condition was also found ($r=0.67$; $p<0.05$). However, a similar daytime correlation was found under non-stressed conditions ($r=0.73$; $p<0.01$) and no correlation between PEA and

creatinine excretion was found at night under either condition. In addition, when data for each subject were accumulated over a 24 hr period, stress increased the amount of PEA (3,052±216 vs. 2,293±237 μg/24 hr; $p<0.05$) but not the amount of creatinine (4.5±0.4 vs. 4.4±0.3 mg/24 hr) excreted, and the amounts of PEA and creatinine excreted per 24 hr were not significantly correlated with one another ($r=0.3$; $p<0.05$). Finally, no significant correlation between any other parameter and the amount of PEA excreted over either a 24 hr or a 12 hr period under either condition was obtained.

DISCUSSION

Our laboratory [12] has previously shown that stress in humans elevates the urinary excretion of endogenous PEA in a manner that is not attributable to changes in diet, or using creatinine excretion as a rough index [5], in renal glomerular filtration rate (GFR). In addition, since urine pH was not decreased by the stressor, the increased PEA excretion could not be attributed to protonation of the substance's amine group and a consequent reduction in PEA reabsorption within the kidney. The data of the present study indicate that cold-restraint stress applied to rats also increases the urinary excretion of endogenous PEA. They also suggest that such increases are likewise not due to stress-associated changes in diet, GFR or urine pH since: stress significantly altered PEA excretion but not urine pH, creatinine excretion or food consumption; and, based upon the fact that PEA and creatinine excretion were generally uncorrelated with one another, the variables responsible for alterations in PEA and creatinine excretion appear to be dissimilar. However, urinary PEA excretion in unstressed rats, unlike that in humans [12], appears to be subject to a diurnal variation. This nocturnal augmentation in PEA excretion is most probably secondary to a dietary increase in the ingestion of PEA or its amino acid precursor [23], phenylalanine, and/or to an increase in GFR and thus blood flow to the kidney. Nevertheless, in general, the rat appears to represent a reasonable model for stress-induced alterations in human PEA disposition.

The mechanism responsible for stress-induced urinary PEA increases in rats, like those in humans [12], is unclear. However, it seems likely that the increased urinary concentration of PEA results secondarily from an increased amount of blood-borne PEA reaching the kidney. This hypothesized elevation in circulating PEA could be derived from a stress-induced activation of the adrenal medulla with a resultant increase in PEA release since this tissue contains a high concentration of PEA [16]. It could also be contributed to by brain since this tissue normally contains PEA and PEA readily passes the blood-brain barrier (e.g., [9]). Alternatively, PEA is known to be rapidly deaminated by the B-form of monoamine oxidase (MAO; [21,24]) and Glover *et al.* [7] have reported that cold-restraint stress in rats results in the increased presence of an unknown non-monoaminergic substance in urine with MAO-inhibitor activity. Consequently, the putative stress-induced elevation in plasma PEA could be due not only to an increase in the release of PEA into blood, but also to an inhibition of PEA metabolism. Regardless of the mechanism(s) responsible for the increases in urinary PEA associated with stress, the present study provides additional support for the hypothesis that the similarities between the consequences of stress and amphetamine administration are due at least in part to PEA. More direct evidence for the functional importance of stress-induced changes in PEA disposition, however, will depend upon future studies concerning the magnitude, duration and selective manipulation of such changes in blood and brain, and their relationship to the behavioral psychiatric and cardiovascular sequelae of stress.

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