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Factors Influencing Plasma Catecholamine Levels in Rats during Immobilization

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DeTURCK, K. H. AND W. H. VOGEL. *Factors influencing plasma catecholamine levels in rats during immobilization.* PHARMAC. BIOCHEM. BEHAV. 13(1) 129–131, 1980.—Catheterized young and old male and female rats in estrus and diestrus were immobilized for 30-min periods. Six serial blood samples were drawn over one hour and the plasma was assayed for the catecholamines (CA) norepinephrine (NE) and epinephrine (E). Baseline CA levels were higher in awake young male rats than in asleep males, and still higher in old male rats. Highest basal NE levels were found in diestrus females. Immobilization produced similar elevations in plasma CA for all groups. Peak values occurred at 1–5 min and declined slowly thereafter toward baseline. Repreated stress of young male rats caused habituation as evidenced by diminished peak CA levels during the second and third restraint. Age, time of exposure and day of the cycle in female rats had subtle, though statistically significant, effects on CA levels at various times during and after immobilization and on the total stress response.

Age Chronic indwelling catheter Estrous cycle Immobilization stress Plasma catecholamines Rats

IMMOBILIZATION has been shown to substantially increase plasma norepinephrine (NE) and epinephrine (E) levels in rats [3]. Previous experiments, however, concentrated on a single restraint of young male animals. In the present study, the effects of repeated restraint, as well as age, time of the day and day of the cycle in female rats on plasma NE and E levels were investigated.

Plasma catecholamine levels were measured radioenzymatically [4] with plasma sampled repeatedly from undisturbed Wistar rats via a chronic venous catheter. The flexible catheter was implanted in the external jugular vein, according to the method by Upton [5] as modified by Pashko and Vogel [2], and the animals were allowed 48 hr for recovery before the first stress. Restraint (except in rats stressed at 10:00 p.m.) was imposed by securely taping the rat to the workbench for 30 min beginning at 10:00 a.m. Six 0.25 ml blood samples were drawn at time 0 (homecage), at 1 min, 5 min, 15 min and 30 min into the stress, and at 60 min (30 min after the animal had been returned to the home cage). Plasma was separated and frozen at −70°C prior to assay and the red blood cells were resuspended in saline and returned to the animal.

In the first experiment, five 3-month-old, male rats weighing approximately 300 g were restrained three times, each trial separated by one day of rest (Table). During the first restraint period, basal NE levels increased rapidly and peaked at 5 min. Thirty minutes after the restraint, NE levels had declined markedly but were still significantly higher than resting levels. Basal E levels demonstrated a greater increase by 1 min and also had higher peak levels at 5 min into the stress. Again, 30 min after restraint E levels had declined but were still significantly higher than resting levels. The second restraint period showed a trend toward smaller increases which were significant for NE at 5 min into the second and third exposure and for E at 5 and 15 min into the third exposure. Thus, habituation seemed to occur early, even to a stressor as severe as restraint. This decrease in NE and E levels correlated well with the observations of less behavioral excitement and decreases in bolus expulsion during the second and third exposure.

An interesting finding was that the rat which showed the greatest elevation in plasma NE and E at the first restraint continued to demonstrate relatively high levels during the second and third periods. Likewise, the rat with the lowest initial stress response showed relatively low catecholamine levels during subsequent restraint periods. The curves for these two rats are shown in the figure.

As the previous stress trials were performed during the normal period of sleep for the rat, an experiment was performed at 10:00 p.m. on a group of five, 3-month-old male rats to examine the effects of time of day on the stress response (Table). Baseline levels of both NE and E were elevated at 10:00 p.m.; however, catecholamine concentrations measured during and after the single night time restraint generally matched the data from the first daytime restraint, although percentage increases over baseline were smaller and baseline levels (60 min) were reached sooner.

It has been reported that plasma NE levels in man increase with age and show a greater increase in old patients after exercise [1]. In five 2-year old rats (life span approximately 2.5 to 3 years), basal plasma concentrations of NE and E were higher, peaked sooner (at 1 min) and tended to decline slower after peak values. In addition, the maximal rise in plasma E concentration was the highest observed in our studies.

The last experiment employed regularly cycling 3-month-old female rats as determined by daily microscopic examination of vaginal smears during the customary two-

TABLE 1

PLASMA NOREPINEPHRINE AND EPINEPHRINE LEVELS IN RATS DURING SINGLE AND MULTIPLE RESTRAINT PERIODS (RP)

pg/ml (percentage increase over baseline)

		Time in min					
Norepinephrine		0	1	5	15	30	60
Young male	RP-1	187 ± 40	763 ± 230* (308)	1265 ± 334* (576)	910 ± 252* (387)	613 ± 77* (228)	359 ± 65* (92)
	RP-2	245 ± 53	716 ± 228* (192)	854 ± 211*† (248)	852 ± 164* (248)	866 ± 275* (253)	414 ± 93* (69)
	RP-2	223 ± 36	662 ± 472 (197)	815 ± 109*† (265)	839 ± 224* (276)	852 ± 334* (282)	546 ± 330 (145)
Young male	(PM)	290 ± 28†	683 ± 92* (136)	1274 ± 158* (339)	758 ± 101* (161)	478 ± 48* (65)	317 ± 30 (9)
Old male		296 ± 48†	1222 ± 234*† (313)	718 ± 77*† (164)	542 ± 109*† (83)	548 ± 62* (85)	684 ± 90* (13)
Female	estrus	160 ± 37	474 ± 114* (196)	1293 ± 160* (708)	1160 ± 194* (625)	708 ± 35* (342)	279 ± 19* (74)
	diestrus	320 ± 31†	527 ± 62* (65)	1473 ± 345* (360)	1263 ± 140* (295)	780 ± 99* (144)	525 ± 100* (64)
Epinephrine		0	1	5	15	30	60
Young male	RP-1	101 ± 29	814 ± 644 (706)	1230 ± 513* (1118)	818 ± 243* (710)	540 ± 189* (435)	288 ± 137* (185)
	RP-2	174 ± 83	583 ± 371 (235)	666 ± 355* (283)	747 ± 277* (329)	764 ± 448* (339)	238 ± 111 (37)
	RP-3	181 ± 99	619 ± 560 (242)	606 ± 173*† (234)	518 ± 163*† (186)	545 ± 399 (206)	225 ± 131 (24)
Young male	(PM)	174 ± 19†	456 ± 75* (162)	1122 ± 278* (545)	744 ± 150* (327)	401 ± 127* (130)	204 ± 27 (17)
Old male		269 ± 59†	1773 ± 412*† (559)	842 ± 163* (213)	826 ± 211* (207)	794 ± 158 (195)	484 ± 184 (80)
Female	estrus	164 ± 39	491 ± 136* (199)	1310 ± 202* (699)	894 ± 140* (445)	691 ± 55* (321)	288 ± 132 (76)
	diestrus	145 ± 37	394 ± 83* (172)	1128 ± 107* (678)	867 ± 171* (498)	552 ± 106* (281)	217 ± 92 (50)

Values represent mean ± standard deviation.

*Values differ from time zero at $p<0.05$.

†Values differ from young males first restraint at individual times $p<0.05$.

Groups of male and female rats consisted of 5 and 4 animals, respectively.

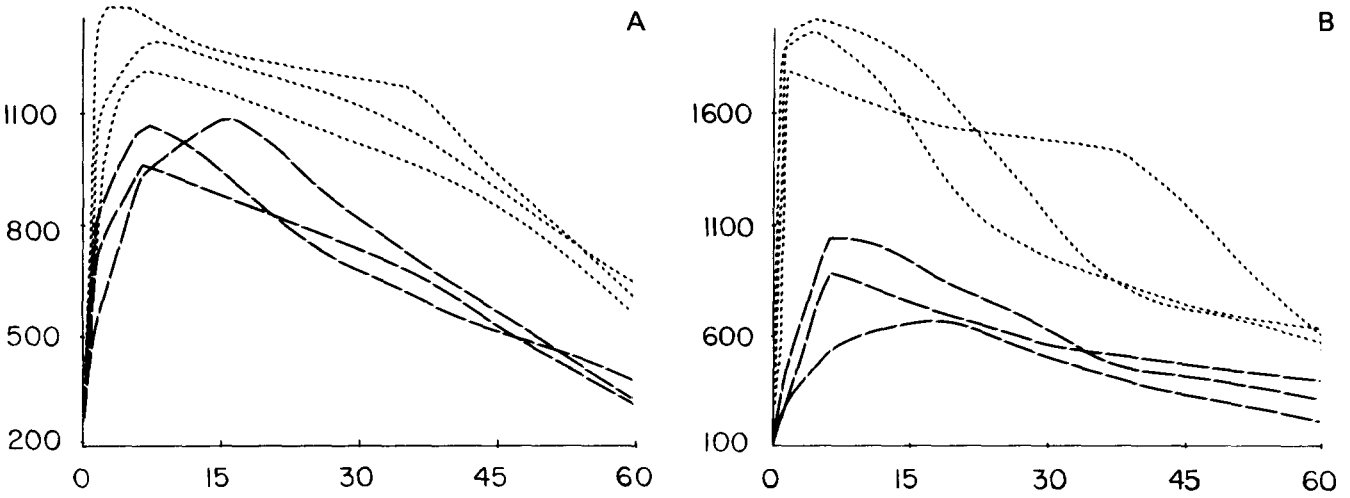

FIG. 1. Plasma NE and E levels (pg/ml) vs time (min) measured during 3 periods of restraint in 2 rats. Figure 1A represents NE levels and Fig. 1B represents E levels. (Rat 1 ----; Rat 2 – – –).

week acclimation period prior to surgery. All catheterizations were performed on rats in diestrus causing no interruption in the estrous cycle. After surgery, four animals were restrained in the succeeding estrus period and four were stressed in the succeeding diestrus period. Baseline NE levels in the distrus rats were twice those measured in the estrus rats and significantly higher than values for male rats of similar age. On the other hand, basal E levels in the estrus and diestrus females were similar to levels found in the young resting males. During restraint, the female rats tended to have a slower increase in both NE and E levels but peak levels and return to baseline were similar to the young males at first exposure.

A survey of all NE and E data shows that basal levels have relatively small standard deviations, whereas these deviations increased markedly during and after immobilization. Thus, individual differences which are not obvious during resting states become readily apparent during periods of stress.

A calculation of percentage increase over basal levels, as shown in the table, shows some interesting trends ($0.05<p<0.1$), but no statistically significant differences due to large standard deviations. A comparison of the areas under the catecholamine-time curves as measured by graphing, cutting and weighing shows no significant differences among the groups with the exception of a diminished stress response in young rats tested at 10 p.m. (total norepinephrine: $p<0.01$ and total epinephrine: $p<0.05$) and old rats (total norepinephrine: $p<0.01$).

REFERENCES

1. Palmer, G. J., M. G. Ziegler and C. R. Lake. Response of norepinephrine and blood pressure to stress increases with age. *J. Geront.* **33:** 482–487, 1978.
2. Pashko, S. M. and W. H. Vogel. Factors influencing the plasma levels of amphetamine and its metabolites in catheterized rats. *Biochem. Pharmac.*, in press, 1979.
3. Popper, C. W., C. C. Chiueh and I. J. Kopin. Plasma catecholamine concentrations in unanesthetized rats during sleep, wakefulness, immobilization, and after decapitation. *J. Pharmac. exp. Ther.* **202:** 144–148, 1977.
4. Upjohn Co., Cat-a-Kit.
5. Upton, R. A. Simple and reliable method for serial sampling of blood from rats. *J. Pharm. Sci.* **64:** 112, 1975.