ACTIVATION OF AUTONOMIC NERVOUS SYSTEM DURING EXPOSURE OF RATS TO RESTRAINT AND WATER IMMERSION STRESS. II. COMPARISON WITH RESTRAINT STRESS

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(Received March 16, 1979)

To determine whether initial sympatho-adrenal stimulation during stress was responsible for inducing a parasympathetic stimulation, the autonomic nervous activities were estimated in rats subjected to either restraint stress or restraint and water immersion stress. The parasympathetic and sympatho-adrenal activities were estimated functionally by measuring responses of gastric motility and heart rate, and also chemically by measuring catecholamine content in urine and tissues such as the heart and adrenals. Restraint and water immersion produced a definite parasympathetic predominance in contrast to the case of restraint alone. But both kinds of stress developed an increase in the sympatho-adrenal activity, and the extent of their increase was almost the same when evaluated on the basis of urinary catecholamine content. These findings indicate that initial sympatho-adrenal stimulation is not directly associated with parasympathetic stimulation. A marked fall in body temperature during stress was assumed to participate somewhere in the induction of parasympathetic stimulation.

Keywords—stress; autonomic nervous system; heart rate; gastric motility; adrenal catecholamines; urinary catecholamines; gastric erosion

It is widely accepted that stress generally stimulates the parasympathetic¹⁻⁵⁾ as well as the sympathetic division of the autonomic nervous system.^{1,2,6-8)} A formerly presumed mechanism on parasympathetic stimulation, to our knowledge, is that initial sympatho-adrenal stimulation elicits a reciprocal or counter stimulation of the parasympathetic division.^{1,2,9)} However, details of such parasympathetic stimulation remain to be elucidated further, for it is not definitely known whether the parasympathetic stimulation invariably occurs with any kind of stress procedure. We have demonstrated that exposure of rats to restraint and water immersion stress stimulates the parasympathetic division, as assessed by measuring gastric motility, following a sympatho-adrenal stimulation.¹⁰⁾ But we have exhibited that restraint alone, could not increase gastric motility in a marked degree comparable to the former stress.¹¹⁾

On the other hand, several investigators have so far reported that restraint stress causes a sympatho-adrenal stimulation.^{7,8)} These facts suggest a difference in the extent or pattern of the parasympathetic stimulation among various kinds of stress procedures, as was the same for sympatho-adrenal stimulation.¹²⁾

The present study was conducted to confirm the validity of the parasympathetic stimulation depending on a sympatho-adrenal stimulation which was demonstrated by Hübner, *et al.*,²⁾ by comparing the two kinds of stress procedures under the same experimental situations.

MATERIALS AND METHODS

Male Wistar rats weighing 220—300 g were used. They were starved for 18 hr but were allowed free access to water. Animals were anesthetized with ethylether, then the abdomen was opened to

introduce an intragastric balloon and an intraperitoneal thermister element, as previously described.¹⁰⁾ Gastric motility was recorded on a kymograph *via* the balloon, and body temperature was monitored *via* a thermister (Shibaura Electronics, MGA-3). The position of the abdominal incision was sutured and then covered with collodion. Heart rate was measured by counting R waves on the electrocardiogram lead II with a biophysigraph (San-ei, SYS-130) connected to a pen writing recorder (San-ei, Type W-809).

Animals were divided into three groups, that is, unstressed controls, restrained rats, and restrained and water-immersed rats. Each control animal was subjected to laparotomy, and placed in a metabolic cage for urine collection after recovery from anesthesia without measuring gastric motility or body temperature. The restraint group of rats were individually put into a restraint cage and kept in a room at 22–23° for 3 or 6 hr. The restraint and water immersion group of rats were similarly restrained and then vertically immersed in water at 25° to the level of the xiphoid process for 3 or 6 hr according to Takagi, *et al.*¹³⁾ Collection of urine in both stressed groups was made through a rubber tube, one end of which was attached to the external part of the genitals with an adhesive (Aron alpha, Toa Chemicals) and the opposite end was connected with a sample bottle containing 2–4 ml of 0.8 N perchloric acid and 0.2% sodium metabisulfite.

After 3 or 6 hr of stress period, the stomachs

of animals were removed and inflated with 10 ml of saline. They were placed in 0.5% formaline for 10 min, opened along the greater curvature, and examined for the presence of gastric erosions. The severity of gastric erosions was calculated as the sum of the length (mm) of each erosion per stomach.

Contents of epinephrine (E) and norepinephrine (NE) in the adrenals, whole brain, heart, glandular stomach, and urine sampled at the end of stress were fluorometrically determined.^{14,15)} Procedures for preparing specimens were the same as described previously.¹⁶⁾

Student's *t* test was used to determine the statistical significance.

RESULTS

1. Formation of Stress-Induced Gastric Lesions

The incidence and the severity of gastric lesions are presented in Table I. No gastric lesion was noted in unstressed controls. The severity of gastric lesions observed after restraint and water immersion stress was much greater than that observed after restraint alone.

2. Patterns of Stress-Induced Gastric Motility

When animals were exposed to restraint and water immersion, an increase in gastric motility occurred with a delay of 156 ± 5.4 min (mean $\pm$ S.E. of 14 animals) after the beginning of the stress procedure. Characteristic features of such gastric motility consisted of a periodical rise in gastric tone, an augmentation in amplitude of gastric con-

TABLE I. Formation of Stress-Induced Gastric Lesions

Groups	After 3 hr of stress		After 6 hr of stress	
	Erosion severity ^{a)} (mm)	Incidence ^{b)}	Erosion severity ^{a)} (mm)	Incidence ^{b)}
Control	—	—	0.0 $\pm$ 0.0	0/6
Restraint	2.5 $\pm$ 0.6	7/9	1.4 $\pm$ 0.6	5/7
Restraint and water immersion	6.8 $\pm$ 1.0 ^{c)}	8/8	15.9 $\pm$ 2.1 ^{d)}	6/6

a) Each value is expressed as the mean $\pm$ S.E.

b) Number of rats with gastric erosions/number of rats per group.

c) $p < 0.01$, when compared with the restrained group.

d) $p < 0.001$, when compared with the restrained group.

traction, and an increase in frequency. No alteration in patterns of gastric motility in restrained rats was observed during the period of 6

FIG. 1. Heart Rate and Body Temperature in Stressed Rats

R+WI; restraint and water immersion stress. R; restraint stress. Each value represents the mean $\pm$ S.E. of the animal number which is shown in parentheses after the individual group. Statistical significance was evaluated at the level of $p=0.05$; +; significantly different from the restrained group. Values plotted at '0' time mean the ones observed after 3 min of stress.

hr.

3. Body Temperature and Heart Rate in Stressed Rats

Changes in body temperature and heart rate in stressed animals are shown in Fig. 1. Body temperature fell abruptly after exposure of rats to restraint and water immersion stress, and the heart rate was markedly lowered in parallel with the changes in body temperature. In the restrained group, heart rate as well as body temperature continued to increase by degrees after the onset of stress, and became constant 1.5 hr later.

4. Content of Catecholamines in Tissues and Urine in Stressed Rats

Changes in NE content of the heart, stomach and brain from stressed rats are shown in Table II. Both stress procedures produced a gradual decrease in NE content of the heart which became statistically significant after 6 hr of stress. The extent of decrease of NE content was somewhat greater with restraint and water immersion stress. No appreciable change in NE content was seen in the stomach and brain after 6 hr of either stress procedure.

Content of adrenal and urinary catecholamines in stressed rats is illustrated in Fig. 2. Adrenal NE levels were not affected after 3 hr of either stress procedure, but were decreased to a slight but significant degree after 6 hr of restraint stress. Adrenal E levels were significantly reduced after 3 and 6 hr of both kinds of stress, although more greatly after restraint and water immersion. As to

TABLE II. Norepinephrine Content in Heart, Stomach and Brain of Stressed Rats

Specimens	Stress period	Norepinephrine content ($\mu\text{g/g}$) ^{a)}					
		Control	(N)	Restraint	(N)	Restraint and water immersion	(N)
Heart	3 hr	—		0.49±0.05	(8)	0.38±0.07	(6)
	6 hr	0.56±0.09	(6)	0.35±0.04 ^{b)}	(7)	0.24±0.03 ^{b)}	(6)
Stomach	6 hr	0.16±0.03	(6)	0.09±0.01	(7)	0.11±0.01	(6)
Brain	6 hr	0.25±0.03	(6)	0.22±0.03	(7)	0.20±0.01	(6)

a) Each value is expressed as the mean $\pm$ S.E.

b) $p < 0.05$, when compared with the control levels.

FIG. 2. Content of Adrenal and Urinary Catecholamines in Stressed Rats

R+WI; restraint and water immersion stress. R; restraint stress. Each value represents the mean $\pm$ S.E. of the animal number which is shown in parentheses after the individual group. Statistical significance was evaluated at the level of $p = 0.05$: *, significantly different from the control group and †; significantly different from the restrained group. The values served as control after 6 hr of stress were also substituted for those to be control after 3 hr of stress. Broken lines indicate changes from levels assumed to be at '0' time.

the decrease in the amount of total adrenal catecholamines, there was no significant difference between either stress technique. Urinary catecholamine excretion, which was estimated as the amount of E and/or NE, was definitely in-

creased after either stress procedure. The increase in urinary E content was more prominent after restraint and water immersion, whereas that of urinary NE content was greater after restraint stress. Thus, no significant difference in the

amount of total urinary catecholamines was noted between the two kinds of stress procedures.

DISCUSSION

The present study confirmed our previous observations that restraint stress did not induce an increase in gastric motility and that its ability to produce gastric lesions was very weak, as compared with that of restraint and water immersion.¹¹⁾ Some authors, however, have pointed out the contraction of the stomach in rats exposed to restraint stress, although no gastric motility was recorded.^{2, 17)} Increase in gastric motility during restraint and water immersion was attributable to parasympathetic effects, because anticholinergic drugs¹⁰⁾ or vagotomy (unpublished data) completely prevented its initiation. Thus, restraint and water immersion, but not restraint alone, induces a parasympathetic predominance on the basis of gastric motility, which probably results from parasympathetic stimulation according to our previous study.¹⁰⁾

In consistency with observations reported before,^{7, 16)} two types of stress procedures were found to increase the sympatho-adrenal activity. The most important result of this study using three kinds of measurements is that there is no appreciable difference between the two stress procedures in the extent to which the sympatho-adrenal system was stimulated. First, a measurement of heart rate was made because changes in heart rate were considered to reflect the autonomic tone¹⁸⁾ under circumstances that did not cause an appreciable change in body temperature.¹⁹⁾ After restraint stress, heart rate gradually increased and remained high, which led to a suggestion of increased sympatho-adrenal activity during a stress period. However, changes in sympatho-adrenal activity during restraint and water immersion could not be evaluated by this method because of a marked fall in body temperature. Second, catecholamine content of tissues such as heart, stomach, brain and adrenals from restrained rats was compared with that from restrained and water-immersed rats. The NE content of the heart and the E content of the adrenals were significantly decreased after 6 hr of both stress

procedures, although the extent of their decrease was somewhat greater after restraint and water immersion. Finally, urinary excretion of catecholamines after restraint was compared with that caused by restraint and water immersion, because such a measurement was considered to be a suitable indicator for evaluating the sympatho-adrenal activity.⁶⁾ The amount of E in the urine of restrained and water-immersed rats was slightly greater than that of restrained rats, but the reverse was true for the amount of NE. Thus, no statistically significant difference was noted between the two kinds of stress in the total amount of urinary E and NE.

From this study, it is reasonably deduced that initial sympatho-adrenal stimulation may not necessarily induce a parasympathetic stimulation. This conclusion is not consistent with the suggestion of Hübner, *et al.* that preceding sympatho-adrenal stimulation would cause a reciprocal or counter stimulation of the parasympathetic division. In addition, the results obtained here, together with those reported previously,¹⁰⁾ suggest that the parasympathetic stimulation during restraint and water immersion takes place *via* some intrinsic mechanism involved in this nerve division. From the difference in the nature of the stress procedures used, we speculate that a marked fall in body temperature may participate somewhere in such mechanism in stimulating the parasympathetic division.

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