DECREASE OF ACh RESPONSE IN ISOLATED DUODENUM FROM REPEATED COLD STRESSED (SART STRESSED) MICE

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Responses to ACh, pilocarpine, BaCl₂ and KCl in isolated duodenum from SART stressed mice (specific stress caused by alternating rhythm in temperature) were investigated using the Magnus's method, and the results were compared with those seen in non-stressed mice. We found that the ACh response was significantly decreased, while the BaCl₂ response was not decreased in SART stressed mice duodenum, as compared with the non-stressed controls. And it is recognized that pretreatment with adrenergic and anticholinergic drugs inhibited the decrease of ACh response.

These results suggest that SART stressed mice may be unbalanced in the autonomic nervous system.

Keywords —stress; SART stress; ACh response; contractile system in smooth muscle; autonomic nervous system

In SART stressed (repeated cold stressed) mice and rats induced by repeating rhythmical changes of environmental temperature from 24° to 8°, many physiological changes¹⁾ in respiration, heart rate, QRS-time on ECG, body weight, *etc.* were observed. From these changes, SART stressed animals were considered to have symptoms suggestive of an imbalance of the autonomic nervous system. ACh response in the isolated duodenum from SART stressed mice was reported to be remarkably decreased¹⁾. On the other hand, Aonuma *et al.*²⁾ considered SART stress to be a severe stress, based on the findings in the biochemical parameters of the adrenal cortical function in SART stressed rats.

An attempt was made herein to make clear the mechanism involved in the decrease of ACh response in the isolated duodenum from SART stressed mice.

Male mice of ddY strain weighing 18—20 g were exposed to SART stress for 5 days by the same method¹⁾ as reported previously. After stress, 2 cm of duodenum was isolated and responses to ACh, pilocarpine, KCl and BaCl₂ were isometrically tested at 32° using the Magnus's method. Rate of contraction was presented as the percentage to the mean value of the maximum contraction (10⁻³ ACh, 10⁻² BaCl₂) in non-stressed mice duodenum.

As seen in Fig. 1, rate of ACh contraction (%) of SART stressed mice duodenum was significantly lower than that of non-stressed control ($p < 0.05$ —0.001). The maximum contraction seen with 10⁻³ (g/ml) ACh was 37.2% that of the control. Contraction caused by 10⁻⁵—10⁻³ ACh in SART stressed mice duodenum was close to the contraction produced by 4—5 × 10⁻⁸ ACh in non-stressed controls. When the dose—response

FIG. 1. Influence of SART Stress on the Contraction induced by ACh and BaCl₂ in Isolated Duodenum from Mice

○—○ normal, ●—● SART stress. Period of SART stress: 5 days, a): $p < 0.05$, b): $p < 0.01$, c): $p < 0.001$, significant differences as compared to normal mice. No. of mice: 10–15/group.

curves of ACh was plotted on the co-ordinate of $[\log x, \log \{y/(y_{max} - y)\}]$, the line of SART stressed mice tended to shift slightly toward the right, became parallel to the line of non-stressed mice. A point of intersection of the X-axis and the above-mentioned line, which shows the concentration of ACh at 50% contraction, is 1.2×10^{-7} in non-stressed mice and 2.8×10^{-7} in SART stressed mice, respectively. Thus, it is reasonable to speculate that the affinity to ACh was a little decreased in the isolated duodenum from SART stressed mice. Such decrease of response could still be observed a few days after cessation of exposure to the SART stress. The response to pilocarpine was similar to that seen with ACh.

The rate of contraction observed with the same concentration of BaCl₂ was slightly decreased in SART stressed mice duodenum, but the difference was not significant. Maximum contraction in both specimens was the same. BaCl₂ is said to act directly on the contractile system in the muscle. From these findings, the contractile system of the duodenal muscle in SART stressed mice is considered to be essentially intact after the SART stress. The line of BaCl₂ response in the co-ordinate of $[\log x, \log \{y/(y_{max} - y)\}]$, unlike the case of

ACh, almost overlapped with the control line. Thus, the affinity of the SART stressed mice duodenum to BaCl₂ was almost the same as that in the control.

The maximal contraction with KCl was significantly decreased in SART stressed mice duodenum, but the affinity was considered to be almost the same, unlike the results of ACh and pilocarpine (data not shown). BaCl₂ and KCl-contraction were not inhibited by pretreatment with atropine (10^{-9} – 10^{-7} g/ml).

The above results suggest that ACh response is selectively decreased in SART stressed mice duodenum.

This decrease may be due to decrease in body weight following the SART stress, or to withering degeneration of the intestine which ensues from decrease in body weight. Therefore, intake of food and length of intestines were measured. However, the daily ingestion of food was found to increase in SART stressed mice, though body weight decreased during the period of the stress. Wet weights of both small and large intestines from SART stressed mice were heavier than in controls, but the length of the small intestine was much the same as in the control. Thus, it cannot be con-

cluded that the decrease in ACh response in the SART stressed mice duodenum is due to the degeneration of intestine as a result of a poor appetite caused by stress.

To study the cause of this decrease of ACh response, various drugs acting on autonomic nervous system were administered *i.p.* to mice once daily for 5 days during the SART stress, and ACh responses of the isolated duodenum were investigated. Pretreatment with adrenergic drugs such as adrenaline, noradrenaline and isoproterenol, and anticholinergic drugs such as atropine sulfate and scopolamine-N-butylbromide inhibited the decrease of ACh response, but antiadrenergic drugs such as guanethidine and α -methyldopa had no effect. These findings suggest that SART stressed mice may be unbalanced between sympathetic and parasympathetic nervous system in the part of intestines.

From above results, it is proposed that the

remarkable decrease in ACh response in the isolated duodenum from the SART stressed mice may be due to change of ACh-receptor sites on the superficial membrane or the neighboring inner layer of muscle of small intestines, and that the contractile system of the muscle itself may be quite intact. However, further studies are required to explain our interesting findings on the decrease of ACh response in the isolated SART stressed mice duodenum.

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