Changes of Total Acetylcholine Content and the Activity of Related Enzymes in SART (Repeated Cold)-Stressed Rat Brain and Duodenum

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Abstract—In SART-stressed rats regarded as pathologically diseased model animals with vagotonic-type autonomic imbalance, a decrease of total acetylcholine (T-ACh) content and enhancements of choline acetyltransferase (CAT) and acetylcholinesterase (AChE) activities were recognized in the basal ganglia and hypothalamus. In contrast, in the duodenum, an increase in T-ACh content and a decrease in AChE activity were found, while CAT activity showed no change. These findings suggest that in both brain areas of basal ganglia and hypothalamus in SART-stressed rats, ACh neurons may be activated.

The changes occurring in brain catecholamines in various stressed animals have been closely studied by numerous investigators. However, there are few reports concerning the relationship between acetylcholine (ACh) and stress.

SART (specific alternation of rhythm in environmental temperature)-stressed (repeated cold-stressed) animals developed by us (1) showed subsensitivity to ACh in the duodenum (2), and the subsensitivity was inhibited by bilateral subdiaphragmatic vagotomy but not by splanchnic sympathectomy (3). In the SART-stressed mouse duodenum, a decrease in the number of muscarinic ACh receptors was found in a binding assay using 3H-labeled QNB (4). From the viewpoint of systemic symptoms, SART-stressed animals are pathologically diseased animals in an autonomically imbalanced vagotonic-type state (5).

From the above-mentioned observations, it is suggested that subsensitivity to ACh in SART-stressed animal duodenum may be associated with functional change in the parasympathetic center. In the present study, therefore, we attempted to examine three cholinergic parameters, total ACh (T-ACh) content and the activities of choline acetyltransferase (CAT) and acetylcholinesterase (AChE) in SART-stressed rat brain and duodenum.

The experimental animals used were male Wistar rats weighing 200–300 g. For the bioassay of ACh content, male guinea-pigs of the Hartley strain weighing about 300 g were used. Biochemical, special-grade reagents were used in all assays. For the assay of the CAT activity, 3H-labeled acetylcoenzyme A (0.55 Ci/mmol, Amersham) was used. For producing SART-stressed animals, the rats were alternately kept at 24°C and −3°C at 1-hr intervals from 9 a.m. to 4 p.m. and then at −3°C from 4 p.m. to 9 a.m. the following morning. These procedures were continued for 5 consecutive days.

Rats used for brain T-ACh content determination were sacrificed by overhead irradiation with 2,450-MHz microwaves at 5 kW for 1.2 sec using a microwave applicator (Toshiba, TMW-6402A). Following decapitation, the brain was isolated and divided into various areas, according to the method of Gispen et al. (6). The extraction of ACh was carried out by the formic acid-acetone technique of Toru and Aprison (7).
Duodenum ACh was extracted according to the method of Yagasaki et al. (8). The T-ACh content in samples obtained from both brain and duodenum was determined by the bioassay technique using isolated guinea-pig ileum according to Blaber and Cuthbert (9) and an isotonic transducer (Nihon Kohden, TD-112S).

CAT activity was assayed according to the radiochemical method of Fonnum (10) using \(^3\)H-labeled acetyl-coenzyme A. Measurement of AChE activity was carried out by the photometric method of Ellman et al. (11) using a spectrophotometer (Hitachi, 100-10).

Figure 1 indicates changes of T-ACh content in SART-stressed rat brain and duodenum. SART-stressed rats showed significant decreases in the T-ACh content of basal ganglia and hypothalamus, but not whole brain and cerebral cortex. The decrease in the hypothalamus, in particular, was considerable. Respective T-ACh values of basal ganglia and hypothalamus were 19.86±0.74 and 15.39±1.07 nmol/g tissue for SART-stressed rats, compared with 25.05±0.97 and 24.90±1.06 for non-stressed rats. On the other hand, duodenum T-ACh was significantly increased from 24.20±0.79 in the non-stressed group to 28.14±0.84 in the SART-stressed group. In addition, the time course of the change in T-ACh content in mouse duodenum during and after the period of stressing corresponded to that in the ACh response in isolated mouse duodenum (12) (data not shown).

Figure 2 shows the activities of related enzymes in rats. As seen in Fig. 2a, in SART-stressed rats, significant increases in CAT activity in the basal ganglia and hypothalamus, which showed changes in T-ACh content, were observed, although little change was noted in the enzyme activity of other brain areas and duodenum.

As shown in Fig. 2b, AChE activity in SART-stressed rats was significantly enhanced in basal ganglia and hypothalamus, where changes in T-ACh content and CAT activity were recognized. By contrast, a significant decrease was observed in this enzyme activity for the duodenum.

From the above-described results, it was found that an inverse correlation existed between T-ACh content and AChE activity. CAT activity, however, did not appear to show any set correlation with T-ACh content.

In addition, we examined the similar cholinergic profile for cold-stressed rats which were reared in a room at -3°C all day long (data not shown). Significant decreases in T-ACh content in cold-stressed rat basal ganglia and hypothalamus were noted, although the rate of these changes was less than that in SART-stressed rats, and little change was recognized in CAT and AChE.

![Fig. 1. ACh content in SART-stressed rat brain and duodenum.](image-url)
activities in cold-stressed rats. Accordingly, the remarkable changes in T-ACh content and the activities of CAT and AChE found in SART-stressed rats may be characteristic of this stress.

In addition to the enhancement of CAT activity, the decrease in T-ACh content of both basal ganglia and hypothalamus following SART stress loading suggests the possibility either that an increase of ACh release or metabolism above the level of synthesis may occur or that the turnover rate of ACh may be enhanced.

In conclusion, it is suggested that the ACh neurons may be activated in SART-stressed rat brain, especially in the hypothalamus and to a secondary degree in the basal ganglia.

An increase in T-ACh content was observed in SART-stressed rat duodenum in contrast to the brain. It is indistinct whether this increase was caused by the decrease in AChE activity, a decrease in ACh release, or others. In the joint study with Dr. Kadota using an electron microscope, high electron-density over all presynaptic nerve terminals and large populations of clear synaptic vesicles were observed in Auerbach’s plexus in SART-stressed rat duodenum (K. Kadota et al., unpublished observation).

The result that opposite changes of both T-ACh content and AChE activity were seen in the brain and duodenum of SART-stressed...
rats is very interesting and important in considering the relationship between these two organs. To clarify these observations, we are currently pursuing further research on vagotomized rats, although the participation of other factors such as chemical transmitters and humoral factors such as hormones and autacoids, should also be investigated.

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