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BRIEF COMMUNICATION

Streptozotocin-Induced Diabetes in Mice Reduces the Nociceptive Threshold, as Recognized After Application of Noxious Mechanical Stimuli But Not of Thermal Stimuli

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KAMEI, J., Y. OHHASHI, T. AOKI AND Y. KASUYA. *Streptozotocin-induced diabetes in mice reduces the nociceptive threshold, as recognized after application of noxious mechanical stimuli but not of thermal stimuli.* PHARMACOL BIOCHEM BEHAV **39**(2) 541–544, 1991.—We report herein that streptozotocin (STZ)-induced diabetes selectively alters the nociceptive threshold with respect to noxious mechanical stimuli. Mice were rendered diabetic by an injection of STZ (200 mg/kg, IV). In the tail-pinch test, the latency of the biting response to forceps was significantly decreased in animals with diabetes of 2 weeks and 8 weeks duration as compared to that in age-matched controls. However, the nociceptive threshold, as determined by the tail-flick test, was not significantly altered. The level of substance P in the spinal cord was significantly increased in mice that has been diabetic for 2 weeks, while, there was a significant decrease, as compared to control levels, in level of substance P in mice diabetic for 8 weeks. However, the level of somatostatin was not significantly altered in mice diabetic for either 2 weeks or 8 weeks. These data suggest that STZ-induced diabetes selectively alters a neuronal system that involves substance P but not somatostatin in the spinal cord.

Diabetes Substance P Somatostatin Nociception

SUBSTANCE P (SP) and somatostatin (SST) have been suggested to be important neurotransmitters in primary sensory neurons involved in nociception (1–3, 5, 8, 14, 15). Kuraishi et al. (5) and Morton et al. (8) suggested that application of noxious mechanical and thermal stimuli specifically increases the release of immunoreactive SP and SST, respectively, from the dorsal horn, an indication that nociceptive mechanical and thermal primary afferents contain SP and SST, respectively.

There is evidence that nociceptive transmission is enhanced in animals with experimentally induced diabetes (4). Previously, we reported that rats with streptozotocin-induced (STZ-induced) diabetes exhibited supersensitivity to SP in the spinal cord, and this supersensitivity may be correlated, in part, with the reduction in the threshold for perception of pain (4). Furthermore, animals with diabetes exhibit abnormalities not only in the metabolism of SP but also in that of SST in some organ tissues (6,10). In sensory nerves, such as the vagus and sciatic nerves, transport and/or levels of SP and SST were found to be significantly altered in STZ-induced diabetic rats (6). Thus it is possible that the function of the neuronal systems that contain SST within the spinal cord may also be altered in diabetic animals. However, experimental support for this hypothesis has not yet been provided.

We have now investigated the possible involvement of the neuronal systems that contain SP or SST in the alterations in nociceptive transmission in diabetic mice by a comparative examination of the threshold for pain perception, as determined by the application of thermal and mechanical noxious stimuli. In addition, the levels of SP and SST in the spinal cords from diabetic mice were also examined.

METHOD

Male mice of the ICR strain (Tokyo Animal Laboratory Inc., Tokyo, Japan), weighing about 20 g at the beginning of the experiments, were used. They had free access to solid food (MF; Oriental Yeast Co., Tokyo, Japan) and water in an animal room which was maintained at 22 ± 1°C with a 12-h light/dark cycle.

Induction by Streptozotocin of Diabetes

Animals were rendered diabetic by an injection of streptozotocin (STZ; 200 mg/kg, IV) prepared in 0.1 N citrate buffer at

TABLE 1

LATENCY OF THE RESPONSES IN THE TAIL-PINCH AND TAIL-FLICK TESTS IN MICE WITH STREPTOZOTOCIN-INDUCED DIABETES

	Tail-Pinch Latency (s)		Tail-Flick Latency (s)	
	Controls	Diabetics	Controls	Diabetics
After 2 weeks of diabetes	2.3 ± 0.3 (23)	1.4 ± 0.2* (26)	7.1 ± 0.3 (21)	7.0 ± 0.3 (22)
After 8 weeks of diabetes	2.3 ± 0.2 (13)	1.2 ± 0.1* (12)	6.6 ± 0.6 (12)	7.1 ± 0.9 (11)

Data are shown as means ± S.E. with numbers of mice in parentheses. Significant differences ($p<0.05$) from control values are indicated by asterisks.

pH 4.5. Age-matched control mice were injected with the vehicle alone. The experiments were conducted 2 weeks or 8 weeks after injection of vehicle or STZ. Mice with serum glucose levels above 400 mg/dl were considered diabetic.

Measurement of the Threshold for Pain Perception

The threshold for pain perception was measured by the following two procedures.

Tail-pinch test. Hemostatic forceps (2 kg constant pressure) were applied to the root of the tail, and the latency of the biting response to the forceps was measured.

Tail-flick test. The tail-flick test was performed by measuring the interval between exposure of the tail to a focused source of radiant heat and the time when the mouse flicked its tail, thereby closing the circuit of a photoelectric cell connected to a timer that measured the interval between the stimulus and response.

Measurement of Substance P- and Somatostatin-Like Immunoreactivity (SPLI and SSTLI) in Spinal Cord

Mice were sacrificed by decapitation, and the dorsal halves of each lumbar spinal cord were rapidly dissected. The dissected tissues from 4 mice were homogenized in 10 volumes (vol./wt) of 0.1 M HCl and heated for 15 min at 95°C. After centrifugation at 38,000 × g for 15 min at 4°C, the supernatant was adjusted to pH 7.0 with 1 M Tris, and the precipitated materials were centrifuged at 8,000 × g for 10 min at 4°C. SPLI and SSTLI in the final supernatant were measured by appropriate radioimmunoassays in accordance with the instructions provided by Amersham International plc (Amersham, Buckinghamshire, UK).

Drugs

The following drugs were used in this study. Streptozotocin was purchased from Sigma Chemical Co., St. Louis, MO. Substance P, with N-succinimidyl-3-(4-hydroxy-5-[^{125}I]iodophenyl) propionate (Bolton and Hunter reagent) and (3-[^{125}I]-iodotyrosyl^{11})-Tyr^{11}-somatostatin-14 were purchased from Amersham International plc. Rabbit antisera for radioimmunoassays of substance P and somatostatin were also purchased from Amersham International plc. Other reagents were of analytical grade and were obtained from commercial sources.

Analysis of Data

Data are presented as means ± S.E. Statistical analyses were performed by analysis of variance, with Student's *t*-test or the Cochran-Cox test.

RESULTS

The body weights of diabetic mice (2 weeks after induction of diabetes, 26.7 ± 0.4 g; 8 weeks, 33.4 ± 0.4 g) were significantly ($p<0.01$) reduced when compared with those of controls (2 weeks, 33.6 ± 0.3 g; 8 weeks, 40.7 ± 0.3 g). Serum glucose levels in diabetic mice (2 weeks, 542.8 ± 33.5 mg/dl; 8 weeks, 672.4 ± 23.2 mg/dl) were significantly ($p<0.01$) elevated when compared with those in the control mice (2 weeks, 199.9 ± 4.2 mg/dl; 8 weeks, 189.6 ± 6.9 mg/dl).

Nociceptive Threshold

Table 1 shows the values for each pain threshold, as determined by both the tail-pinch and the tail-flick test, for diabetic and control mice. In the tail-pinch test, animals with diabetes of 2 weeks and 8 weeks duration had lower spontaneous pain-threshold values than did the respective controls ($p<0.05$), as evidenced by a significant decrease in the latency of the biting response to forceps. However, no significant differences in the nociceptive threshold, as determined by the tail-flick test, were seen between the control and diabetic mice at either 2 weeks or 8 weeks after the induction of diabetes.

Levels of SPLI and SSTLI in the Spinal Cord

The results of experiments designed to investigate the influence of diabetes on levels of SPLI and SSTLI in the spinal cord are presented in Table 2. As can be seen from the table, 2 weeks after induction of the diabetes, basal levels of SPLI in the spinal cord of diabetic mice were significantly higher ($p<0.01$) than those in controls. However, by 8 weeks, the levels of SPLI were significantly ($p<0.01$) lower in the diabetic mice as compared with controls. By contrast, there were no significant differences in the levels of SSTLI in the spinal cords of control or diabetic mice at either 2 weeks or 8 weeks after induction of diabetes.

DISCUSSION

The present experiments show that the threshold for pain perception in response to noxious mechanical stimuli was reduced when animals were tested both 2 weeks and 8 weeks after induction of diabetes. This result is consistent with our previous observation that the latency in the tail-pinch test was significantly decreased in diabetic rats (4). However, we did not observe any significant changes in the nociceptive threshold when noxious thermal stimuli were applied. This result indicates that STZ-induced diabetes selectively alters the nociceptive threshold as determined by the application of noxious mechanical stimuli but not as determined by application of thermal stimuli.

TABLE 2

LEVELS OF IMMUNOREACTIVE SUBSTANCE P AND SOMATOSTATIN IN DORSAL SPINAL CORD FROM MICE WITH STREPTOZOTOCIN-INDUCED DIABETES

	Substance P (μg/mg protein)		Somatostatin (μg/mg protein)	
	Controls	Diabetics	Controls	Diabetics
After 2 weeks of diabetes	0.6 ± 0.1 (6)	2.0 ± 0.3* (4)	5.4 ± 0.9 (6)	6.4 ± 0.5 (5)
After 8 weeks of diabetes	7.4 ± 0.2 (5)	6.6 ± 0.1* (6)	22.3 ± 2.9 (6)	22.1 ± 1.8 (6)

Values are means ± S.E. with numbers of samples in parentheses. Each sample was prepared from 4 spinal cords. Significant differences ($p<0.01$) from control values are indicated by asterisks.

In the present study, we observed that mice diabetic for 2 weeks had higher levels of SPLI in the spinal cord than did control mice, but they displayed unaltered levels of SSTLI in the spinal cord. Conversely, mice diabetic for 8 weeks demonstrated a significant decrease, as compared to controls, in the levels of SPLI in the spinal cord, whereas the levels of SSTLI remained unaltered. We were surprised to find that the levels of SPLI in the spinal cord of mice diabetic for 2 weeks were significantly elevated as compared to those of age-matched control mice. The differences in the data obtained for levels of SPLI in the spinal cord of mice diabetic for 2 weeks and 8 weeks may be attributed to the time course of the development of diabetic complications. Sidenius et al. (12) have reported that rats studied a minimum of 4 weeks after the induction of diabetes demonstrated reductions in rates of protein synthesis in dorsal-root ganglia, such a reduction possibly being secondary to reduced uptake of amino acids (13). Our findings support the possibility of reduced synthesis of substance P in dorsal-root ganglia of animals that were diabetic for 8 weeks. We recently reported that, in rats diabetic for 8 weeks, latency in the tail-pinch test was significantly decreased as compared with controls (4). In these animals, specific binding by receptors of ^{3}H-substance P was significantly elevated in the spinal cord, whereas the level of SPLI in the spinal cord was significantly reduced. Accordingly, we concluded that postjunctional supersensitivity to substance P develops in the spinal cord of diabetic animals, and the development of such supersensitivity to substance P may be the cause of the reduction in the threshold for pain perception (4). Thus, it is possible that the mechanism responsible for the reduction in the threshold for pain perception in mice with diabetes of 8 weeks duration is same as in rats. However, the mechanisms responsible for increased levels of SPLI in the spinal cord of animals with diabetes of 2 weeks duration is unclear. It is difficult to ascertain whether the increase in SPLI in the spinal cord of mice diabetic for 2 weeks is brought about by a decrease in the rate of release of SP or by an increase in the rate of its synthesis. However, we have found that the potassium-evoked release of SPLI from the spinal cord of diabetic rats was greater than that in control rats (9). It seems likely, therefore, that the increased levels of SPLI in the spinal cord of mice diabetic for 2 weeks are attributable to an increase in the rate of synthesis of SP. The increase in the rate of synthesis of SP may lead to an increase in the rate of turnover of SP, so that the rate of release of SP is increased. The release of excessive amounts of SP from the spinal cord may be associated with the abnormalities in nociceptive transmission in mice with diabetes of 2 weeks duration. There are some reports that the intrathecal application of SP produced a hyperalgesia (7, 11, 16). The presence of differential response to intrathecal application of SP in mice diabetic for 2 weeks and 8 weeks then our hypothesis might be more clearly provided. Furthermore, we cannot deduce any details of the mechanisms responsible for the opposite directions of changes in the levels of SPLI during the course of the diabetes at present. The reason of marked changes in control levels of SPLI and SSTLI in the spinal cord at the 2 and 8 weeks intervals is unclear. We observed that the levels of SPLI and SSTLI in the spinal cord were increased in an age-related manner in both the control and diabetic mice (data not shown). Thus it is possible that the different control levels of SPLI and SSTLI at the 2 and 8 weeks intervals may be due to the discrepancy of age. However, it is also unknown the mechanism underlying this age-related transition. Further studies are needed to resolve these problems.

In conclusion, our present results strongly suggest that STZ-induced diabetes selectively alters neurotransmission that involves SP, but not neurotransmission that involves SST, in the spinal cord. Furthermore, this selective alteration in neurotransmission with respect to SP may be correlated with the selective reduction in the threshold for responses to noxious mechanical stimuli. This suggestion is consonant with previous reports that the nociceptive mechanical and thermal primary afferents contain SP and SST, respectively (1, 2, 5, 8). SSTLI is released in the dorsal horn upon application of noxious thermal stimuli but not upon application of noxious mechanical stimuli (5,8), whereas the release of SPLI in the same region of the spinal cord has been evoked by both types of noxious stimulus (1,2), as well as mechanical noxious stimuli exclusively (5).

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