The Mechanism of Alleviation of the Epileptic Seizures Following the Caudal Excision of the Pancreas : Its Effects on the Brain Excitability, Electrolytes and Brain Carbonic Anhydrase

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INTRODUCTION

In 1951, Tokuoka[29] reported that the caudal excision of approximately two thirds of the pancreas was effective in alleviating the epileptic seizures in 66.7% of 27 patients with genuine epilepsy, the result being better in childhood. On the basis of these observation, he suggested that zinc metabolism could play an important role in neurochemical backgrounds of epileptic seizures.

Since then, in our laboratory, a series of experiments have been performed in order to verify this assumption.[7][18][19]

In studies on the alleviating effect of the caudal excision of the pancreas, it is firstly necessary that changes in brain excitability and the underlying neurochemistry following the operation are experimentally explained.

Concerning carbonic anhydrase, it has been shown that the enzyme contains zinc, and that brain carbonic anhydrase is closely related to brain excitability.[3][9][12][17][21]

On the other hand, Fuchimoto et al. (1966)[7] have indicated that zinc may affect seizure mechanisms through the action on brain electrolytes, on the basis of the experimental findings that zinc injected systemically increases brain excitability and causes changes in electrolyte distribution, and that intracerebral injection of zinc evokes tonic extensor seizures, which are completely protected by calcium ion.

Therefore, this experiment was undertaken in an attempt to establish the effect of the caudal excision of the pancreas on brain excitability, brain carbonic anhydrase and electrolyte distribution.

MATERIAL AND METHODS

Adult male mice of dd strain were used. The animals, weighing 17-19 gm at the beginning of the experimental periods, were kept at the constant temperature of 20-22°C. and food and water were freely offered during the experimental

periods.

Treatment of animals.

Caudal excision of the pancreas was performed as described below. Under light ether anesthesia, the upper median abdomen was incised. The stomach was grasped with blunt forceps and retracted to expose the mobile pancreas, which was ling between the duodenal "C" loop on the right and the spleen on the left. The splenic artery was ligated just distal to the superior mesenteric artery, and the distal portion of the gland was removed. This resulted in excision of approximately two thirds of the pancreas. The abdomen was closed in one layer. The animals were sacrificed for experiments 3 weeks and 6 weeks after pancreatectomy. Control mice were given only a laparotomy under anesthesia. Each of 13 mice in each group was weighed once weekly for 6 weeks after the operation and macroscopic findings were observed.

Blood sugar level.

Blood sugar level following pancreatectomy was measured by the method of Sasaki (1964),[24] which needed only 20λ of serum.

Determination of brain excitability.

Two measures of brain excitability were adopted, the seizure threshold and the intensity of seizure. Electroshock seizure threshold (EST) and duration of the maximal electroshock seizure (MES) were respectively measured using Woodbury and Davenport's apparatus (1952),[35] by which the interelectrode electric current can be changed independently of the resistance of the experimental animals. By means of a corneal electrode, 60 cycle/sec. alternating current was applied for 0.2 sec. For determination of the EST, the stimulus was delivered three times a week and EST was measured 3 and 6 weeks after pancreatectomy. According to Takahashi et al. (1961),[27] EST was defined as the electric current to evoke clonic movements involving the head and forelegs, and was measured to within 0.2 mA. MES was induced by currents of 50 mA, and the duration of the tonic flexion phase of the hindlegs (TF), the tonic extension phase of the hindlegs (TE) and the clonic phase of the entire body (CL) were measured.

Water and electrolyte analyses.

All animals were sacrificed by decapitation without anesthesia. For measurement of water content, brain sample was dried at 105°C. to a constant weight and water content was calculated from the difference between wet and dry weight. Electrolyte analysis was done with the utmost care to prevent contamination. All glass implements were sufficiently washed by ion-free-water after soaking in 6N HNO_3 for at least 24 hours. For determination of brain electrolytes, two series of experiments were performed. In a series of experiments, fresh whole brain was homogenized with 8 ml of 10 % W/V trichloroacetic acid. After extraction for at least 1 hour and centrifugation at 800**g** for 10 minutes, the supernatant obtained was analyzed for sodium, potassium and calcium concent-

ration. In another series of experiments, fresh sample of cerebral hemisphere was dried at 105°C. to a constant weight, and electrolytes were extracted with 0.1N HNO_3 for at least 3 hours. The extracts were analyzed for sodium, potassium and chloride concentrations. Serum electrolyte concentration was measured as described below; the animals were sacrificed by decapitation without anesthesia and the spouting carotid blood was withdrawn. Serum was obtained by centrifugation with capillary tubes of 1.5 mm in diameter. With non-hemolytic serum, sodium, potassium and chloride concentrations were measured. Electrolyte concentrations of brain and serum samples were measured for sodium and potassium by means of Hitachi flame photometer. Calcium was measured by the method of Appleton et al. (1959),[1] and chloride by the method of Schales and Schales (1941).[25] Water content in the brain was expressed as per cent of wet weight. Electrolyte values in the brain were shown as mM/kg wet tissue, and in serum as mM/l.

Intracellular and extracellular electrolyte calculation.

Intracellular and extracellular sodium and potassium distributions were calculated according to the formula of Hasting and Eichelberger (1937).[10] The calculation is based on the two assumptions: (a) that serum ultrafiltrate can be used to measure the concentration of the extracellular electrolytes, and (b) that chloride is an ion confined to the extracellular space. Many investigators[2][11][34] have shown that the latter assumption is also applicable in the brain. In the present study, therefore, the intracellular and extracellular distribution of sodium and potassium in the brain were calculated on the basis of the above assumptions. The changes of the intracellular and extracellular cations were made using the mean values calculated for each group of mice.

Measurement of brain carbonic anhydrase activity.

Of the whole brain and cerebral hemisphere, fresh tissue was homogenized in 100 volumes of ice-cold distilled water. Carbonic anhydrase activity in the homogenate was determined by Nishimura's modification (1963)[21] of Maren's pH changing method (1960)[15] and expressed as unit per 100 mg wet tissue.

RESULTS

1. General findings

Pathological findings such as emaciation, fatness, powerlessness, hypersensitivity, diarrhea etc. could not be detected in pancreatectomized mice. There was no significant change in the growth curves in the each group (Figure 1). In 100 pancreatectomized mice, 8 mice died within three days following pancreatectomy and 3 additional mice died during experimental periods. In 100 control mice, 6 mice died within three days following operation.

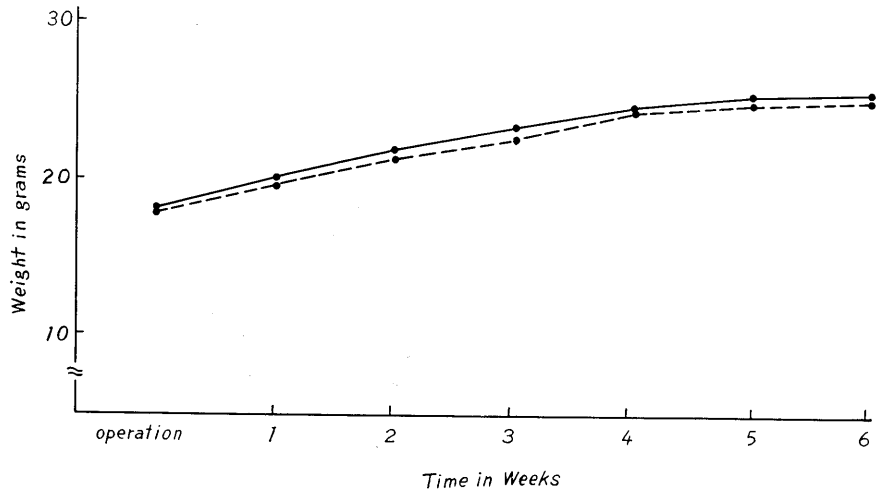

Fig. 1. Body weight following caudal excision of the pancreas
•——• control : average of 13 animals
•·····• pancreatectomized : average of 10 animals

Figure 2 shows the alteration of blood sugar level (24 hours, 48 hours, 4 days, 1 week, 3 weeks and 6 weeks after pancreatectomy). Pancreatectomy did not produce hyperglycemia during experimental periods.

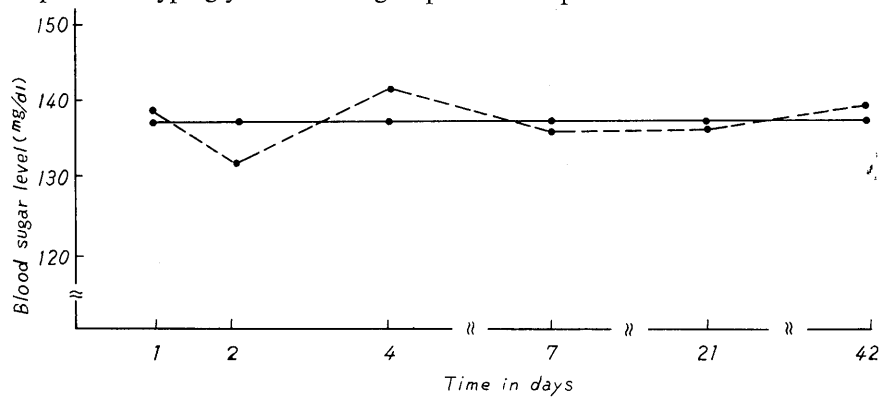

Fig. 2. Change of blood sugar level following caudal excision of the pancreas
•·····• pancreatectectomized : each points are mean value of 5 animals
•——• control : average of 7 animals (136.5 mg/dl)

2. Brain excitabiltiy.

Table 1 shows the effect of pancreatectomy on EST and duration of MES. The EST of the pancreatectomized mice after 6 weeks was 7.2 ± 0.24 mA (mean value ± S. D.) and showed a significant elevation as compared with that of 6.9 ± 0.26 mA of control mice, while the EST in 3 weeks after pancreatectomy remained unchanged. The duration of the tonic extension phase

of the MES was significantly shortened in pancreatectomized mice after 3 weeks and tended to shorten after 6 weeks.

Table 1. Effect of caudal excision of the pancreas on brain excitability

		3 weeks after pancreatectomy		6 weeks after pancreatectomy	
		pancreatectomized	control	pancreatectomized	control
EST (mA)		6.9 ± 0.26 (10)	6.8 ± 0.19 (12)	7.2 ± 0.24 (19)**	6.9 ± 0.26 (20)
MES (sec)	TF	1.3 ± 0.17 (8)	1.2 ± 0.12 (14)	1.4 ± 0.24 (15)	1.3 ± 0.10 (15)
	TE	11.8 ± 1.5 (8)*	13.1 ± 1.2 (14)	12.3 ± 1.2 (15)	12.8 ± 1.8 (15)
	CL	3.0 ± 0.6 (8)	3.3 ± 0.8 (14)	3.9 ± 0.5 (15)	3.7 ± 0.5 (15)

Values are expressed as mean ± standard deviation.
( ): Number of experiments
Significant difference of experimental value from control value
** $P<0.01$ * $P<0.05$

It has been shown that EST is influenced by room temperature and body weight.[26)35)] However, it is obvious that the above changes in brain excitability are due to pancreatectomy, since electrical stimulation was applied to settle weighed mice (21-24g, 3 weeks after operation; 23-26g, 6 weeks) in constant room temperature at 20-22°C.

3. Water and electrolyte analyses in the brain.

Water content and electrolyte concentration in whole brain are presented in Table 2.

Table 2. Water and electrolyte content in whole brain 3 and 6 weeks after caudal excision of the pancreas

		total H_2O %	Na mM/kg	K mM/kg	Ca mM/kg
3 weeks after pancreatectomy	pancreatectmized	77.0 ± 0.11 (5)	46.4 ± 1.6 (8)	100.6 ± 4.2 (8)	1.28 ± 0.20 (8)
	control	76.8 ± 0.11 (5)	45.5 ± 2.0 (8)	101.9 ± 2.1 (8)	1.12 ± 0.12 (8)
6 weeks after pancreatectomy	pancreatectomized	77.0 ± 0.12 (5)	47.9 ± 3.7 (8)	100.9 ± 4.4 (8)	1.36 ± 0.18* (8)
	control	77.1 ± 0.11 (5)	46.5 ± 2.4 (8)	100.9 ± 3.6 (8)	1.17 ± 0.10 (8)

Values are expressed as mean ± standard deviation.
( ): Number of experiments
Significant difference of experimental value from control value
* $P<0.05$

Pancreatectomy did not produce changes in water content and sodium and potassium concentration. But calcium concentration tended to increase 3 weeks after pancreatectomy and showed a significant increase after 6 weeks.

On the other hand, there was no difference in water and electrolytes in cerebral hemisphere (Table 3).

Table 3 Water and electrolyte content in cerebral hemisphere 6 weeks after caudal excision of the pancreas

	total H_2O %	Na mM/kg	K mM/kg	Cl mM/kg
pancreatectomized	78.7 ± 0.25 (10)	48.8 ± 1.9 (9)	101.4 ± 3.3 (9)	30.8 ± 3.6 (10)
Control	78.8 ± 0.44 (10)	48.2 ± 1.6 (12)	101.2 ± 3.6 (12)	30.9 ± 2.6 (12)

Values are expressed as mean ± standard deviation.
( ): Number of experiments

4. Serum electrolyte analysis.

Only a remarkable change in serum electrolytes in pancreatectomy was a significant increase of sodium concentration after 6 weeks ; the concentration was higher by more than 5 mM/l. Potassium and chloride concentration were scarcely changed by pancreatectomy (Table 4). In this case, the propriety of separation of serum using the capillary tubes was studied as follows ; electrolytes of the sample separated with the capillary tubes was compared with those of the sample separated with commonly used test tubes. Consequently, the separation using the capillary tubes seemed to be proper, becauce there was no difference in electrolytes between each series.

Table 4. Change of serum electrolyte concentration 3 and 6 weeks after caudal excision of the pancreas

	3 weeks after pancreatectomy			6 weeks after pancreatectomy		
	Na mM/1	K mM/1	Cl mM/1	Na mM/1	K mM/1	Cl mM/1
pancreatectomized	147.5±4.2 (8)	6.32±0.35 (8)	113.2±3.2 (8)	151.4±4.9** (10)	6.22±0.29 (10)	113.4±3.0 (10)
control	146.3±3.8 (10)	6.29±0.32 (10)	113.7±2.9 (10)	145.7±3.4 (12)	6.36±0.33 (12)	111.5±3.3 (12)

Values are expressed as mean ± standard deviation.
( ): Number of experiments
Significant difference of experimental value from control value
** $P<0.01$

Moreover, the influence of anesthesia on serum electrolytes was studied. In case of unanesthesia, potassium concentration was 6.19 ± 0.26 mM/l (the mean of 5 experiments ± S. D.) and was 25.8 % higher than that of 4.93 ± 0.25 mM/l (the mean of 5 experiments ± S. D.) in case of anesthesia, while there was no difference in sodium and chloride concentration. Therefore, it was considered that the increase of potassium in the unanesthetized group might be produced by exercise of the animals.[8]

5. Intracellular and extracellular electrolyte distribution in the brain.

The ratio of extracellular to intracellular sodium concentration, when calculated on the basis of chloride space as a measure of extracellular fluid volume, was found to be increased in pancreatectomized group, while the ratio of intracellular to extracellular potassium concentration was also found to be increased (Table 5).

Table 5. Intracellular and extracellular distribution of electrolytes in the brain 6 weeks after caudal excision of the pancreas

	chloride space %	mM/kg of fat free tissue[14]				[Na]e / [Na]i	[K]i / [K]e
		[Na]e	[K]e	[Na]i	[K]i		
pancreatectomized	24.9	41.43	1.70	7.65	100.22	5.42	59.0
control	25.4	40.67	1.77	7.81	99.94	5.21	56.5

Values are calculated on the basis of chloride space as a measure of extracellular fluid volume according to Hasting & Eichelberger.[10] Water content of serum assumed to be 92 per cent and specific gravity of serum 1030.
e: extracellular i: intracellular
(See Table 3 and 4 for original data)

Table 6. Brain carbonic anhydrase activity 3 and 6 weeks after caudal excision of the pancreas (unit/100mg)

		cerebral hemisphere	whole brain
3 weeks after pancreatectomy	pancreatectomized	64.2 ± 9.8* (10)	96.3 ± 15.4 (10)
	control	75.9 ± 7.9 (10)	91.8 ± 14.7 (10)
6 weeks after pancreatectomy	pancreatectomized	60.6 ± 11.8* (10)	90.0 ± 14.9 (10)
	control	71.7 ± 9.2 (10)	88.7 ± 10.6 (10)

Values expressed as mean ± standared deviation.
( ): Number of experiments
Signicant difference of experimental value from control value
* $P<0.05$

6. Brain carbonic anhydrase.

Activity of brain carbonic anhydrase is shown in Table 6. Although there was a remarkable difference by individuals in activity of brain carbonic anhydrase, the enzymatic activity of cerebral hemisphere was significantly decreased either

3 weeks or 6 weeks after pancreatectomy. On the contrary, there was no difference in carbonic anhydrase activity of the whole brain.

DISCUSSION

From the present experimental findings, it is evident that subtotal pancreatectomy causes the decrease of brain excitability (i. e., the elevation of EST and the reduction of MES). This change in brain excitability is not due to the disturbance of carbohydrate metabolism, because of lack of recognizable hyperglycemia.

Although the relationship between electrolyte and brain excitability has been discussed by many investigators, it has almost agreed that total sodium and total potassium concentration in the brain do not correlate with brain excitability.[11)16)22)]

Concerning serum electrolyte, serum potassium appears to have a minor influence on brain excitability at best, [6)30)32)] although it is well known that serum potassium concentration is increased after convulsion.

On the other hand, it has been shown that sodium concentration in plasma is intimately correlated with the level of brain excitability of mice as measured by EST[5)31)32)]; an elevation of EST is accompanied by a corresponding increase in plasma sodium concentration and a fall of EST is accompanied by opposite effect, namely plasma sodium and EST rise and fall together.[32)] In addition, in human, Kurosawa et al. (1957)[13)] have reported that serum sodium concentration is decreased when abnormal pattern appears in electroencephalogram by hyperventilation.

Considering from the above reports, it is likely that the increase of serum sodium concentration in pancreatectomized mice causes the elevation of EST.

In the further studies on electrolyte problem, Timiras et al. (1954)[28)] and Woodbury (1954)[33)] have shown particularly that the ratio of extracellular to intracellular sodium concentration in the brain, when calculated on the basis of the chloride space as a measure of extracellular fluid volume, is intimately involved in the brain excitability; an increase in the ratio is associated with a decrease of brain excitability, while a decrease in the ratio is associated with opposite effect. Therefore, the increase in the ratio of extracellular to intracellular sodium concentration in pancreatectomized mice, calculated on the basis of the chloride space, is considered to be responsible for the decrease of brain excitability.

The relationship between brain calcium and seizure susceptibility is not yet established. However, it is quite possible that change in brain calcium may affect brain excitability, because calcium ion is considered to be important in maintaining membrane excitability or regulating the membrane permeability to

sodium and potassium ions in the excitable tissue. But it may be questionable that such a slight increase of brain calcium as observed in pancreatectomized mice is decidedly responsible for the decrease of brain excitability.

On the other hand, it is well known that brain calcium is intimately related to respiration of brain tissue in vitro[23] ; a decrease in the ratio of K/Ca is associated with a decrease of respiration of brain tissue. Moreover, considering that the level of respiration in brain tissue is correlated with brain carbonic anhydrase activity,[4,21] it appears that the increase of brain calcium concentration and the decrease in the ratio of K/Ca are connected with the decrease of brain carbonic anhydrase activity in pancreatectomized mice.

It has been generally proved that brain carbonic anhydrase is intimately correlated with brain excitability; the enzyme is considered to be involved in the regulation of neuronal sensitivity to stimulation by removing excess carbon dioxide as a end product of metabolism.[4] Moreover, it is currently accepted that brain carbonic anhydrase is closely connected with MES, because acetazolamide, carbonic anhydrase inhibitor, reduces the duration of MES without affecting EST.[9,17,20,26] So, the decrease of brain carbonic anhydrase activity in pancreatectomized mice seems to be responsible for the decrease of the duration of MES.

Thus, it may be concluded that the changes in electrolytes and brain carbonic anhydrase are sufficient to explain the neurochemical mechanisms which underlie the changes in EST and MES following subtotal pancreatectomy.

Zinc metabolism and its relationship to brain excitability following subtotal pancreatectomy will be discussed elsewhere.

SUMMARY

The effect of subtotal pancreatectomy on brain excitability and its neurochemical mechanisms were studied in mice.

The results can be listed as follows:

1. Subtotal pancreatectomy decreased brain excitability in mice, as indicated by an elevation of electroshock seizure threshold and shortening of maximal seizure duration.

2. Subtotal pancreatectomy produced alterations in electrolyte concentration in the brain and serum; brain calcium concentration tended to increase after 3 weeks and showed a significant increase after 6 weeks, while brain sodium, potassium and chloride did not change. Serum sodium concentration was significantly increased 6 weeks after pancreatectomy, while serum potassium and chloride concentration were altered only slightly.

3. Subtotal pancreatectomy caused alteration in electrolyte distribution in the brain; the ratio of extracellular to intracellular sodium concentration, when

calculated on the basis of the chloride space as a measure of extracellular fluid volume, was found to be increased, while the ratio of intracellular to extracellular potassium concentration was also found to be increased.

4. Activity of brain carbonic anhydrase was significantly decreased either 3 weeks or 6 weeks after pancreatectomy.

5. From the discussion in this paper, it may be concluded that the increase in serum sodium or extracellular sodium concentration in the brain following subtotal pancreatectomy is responsible for the elevation of the electroshock seizure threshold, while the decrease in carbonic anhydrase activity in the brain is closely related to the reduction of the maximal seizure duration.

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