Medical Journal of the
Islamic Republic of Iran

Volume 7
Number 4
Winter 1372
February 1994

Basic Science In Medicine

SIGNIFICANT CHANGES IN THE ACTIVITY OF GABA-TRANSAMINASE AND SUCCINATE SEMIALDEHYDE DEHYDROGENASE OF MOUSE HYPOTHALAMUS FOLLOWING PERIPHERAL INJECTION OF CHOLECYSTOKININ-8 AND/OR CAERULEIN

R. YAZDANPARAST AND D. QUJEQ*

From the Institute of Biochemistry and Biophysics, University of Tehran, and the
**Dept. of Biochemistry, Tarbiat Modarres University, Tehran, Islamic Republic of Iran.*

ABSTRACT

The activities of 4-aminobutyric-2-oxoglutaric acid transaminase (GABA-T) and succinate semialdehyde dehydrogenase (SSADH) were determined in mouse hypothalamus after peripheral injections of cholecystokinin-8 (CCK-8)and/or caerulein (CLN). GABA transaminase activity was measured utilizing endogenous succinate semialdehyde dehydrogenase to convert the product of GABA-T, succinate semialdehyde, to succinate. The concurrently formed NADH was used as an estimate of GABA-T activity. Similarly, the activity of SSADH was determined in terms of NADH. Injection of CCK-8 and/or CLN inhibited the GABA-T and SSADH activities in dose-dependent responses. The activities of GABA-T and SSADH were diminished by 52 and 66 percent respectively, 30 minutes after injection of CCK-8 (50 µg/kg body weight).Similarly, peripheral injection of CLN (50 µg/kg body weight) reduced the activity of GABA-T and SSADH by 56 and 65 percent respectively, in 30 minutes. Using *in vitro* models, the full activities of GABA-T and/or SSADH, after incubation with CCK-8 and/or CLN, were restored following 30 hours of dialysis at 4°C. These results indicate direct and reversible interactions between the catabolizing enzymes of GABA (GABA-T and SSADH) and the peptides CCK-8 and CLN.

MJIRI, Vol. 7, No. 4, 263-268, 1994.

Keywords: Caerulein, Cholecystokinin-8, Catabolism, GABA-transaminase, Succinate semialdehyde dehydrogenase.

Abbreviations used: AOAA, Aminooxyacetic acid; CCK-8, Cholecystokinin (Sulfated form); CLN, Caerulein; GABA, γ-aminobutyricacid; GABA-T, GABA transaminase; GAD, Glutamate decarboxylase; NAD^+, Nicotinamide adenine dinucleotide; NADH, reduced NAD^+; SA, succinic acid; SSA, Succinate semialdehyde; and SSADH, Succinate semialdehyde dehydrogenase.

Address correspondence to:
Dr. R. Yazdanparast
Inst. Biochem. Biophys.
P.O. Box 13145-1384
University of Tehran
Tehran, I.R. Iran

INTRODUCTION

Cholecystokinin octapeptide (CCK-8), purified from gastrointestinal and central nervous tissues,[1] and caerulein (CLN), a decapeptide closely related to the C-terminal octapeptide of cholecystokinin and isolated from the skin of the frog *Hyla caerulea*,[2] were found to possess various and similar central activities.[3,4]

Using animal models, these physiological activities have been classified as analgesia, antinociception,[6] antistereotypy,[7] satiety,[8] sedation,[9] and hypothermia.[10] Although the exact mechanism(s) of action of CCK-8 and CLN in the central nervous system have not been worked out in detail, based on the enhanced release of GABA from rat cerebral cortex slices, after either CCK-8 or CLN treatment in vitro,[11] or elevation in the threshold for drug-induced convulsions,[12] it has been concluded that CCK-8 and CLN may act upon the central GABAergic system. Additionally, it has been reported that peripheral injection of CLN to rats has altered dopamine turnover in striatum.[13] Since the regulation of the activity of the dopaminergic system is under the influence of GABAergic and glutaminergic system,[14] it is possible that CLN and CCK-8 may modulate the central GABAergic system. In a study conducted by Nagahama,[15] it has become evident that peripheral injection of CCK-8 and CLN to mice caused a reduction in the GABA content of striatum, while the GABA content was increased in hypothalamus and frontal cortex 60 mintues after injection. On the other hand, with CLN and CCK-8, the GABA accumulation after AOAA treatment decreased in striatum and hypothalamus 30 minutes after injection. Although the modulation of the GABAergic system with CCK-8 or CLN is evident from these observations, the reason for different effects of these peptides on GABA content and accumulation in the hypothalamus is not clear and further investigation is required.

In the present study, the acute effects(s) of peripheral injection of CLN and CCK-8 on the activities of two of the catabolizing enzymes of GABA, namely, GABA-T (E.C.2.6.1.19) and SSADH (E.C.1.2.1.16), were investigated using the mouse hypothalamus.

MATERIALS AND METHODS

Materials

GABA, Triton x-100, 2-oxoglutaric acid, SSA, GABASE, 2-mercaptoethanol, sucrose, ethanol, NAD^+, NADH, and Tris-HCl were purchased from Sigma Chemical Company (Paris, France). CCK-8 (sulfated form) and CLN were obtained from Peninsula Laboratories Europe Ltd. (Merseyside, England). CCK-8 and CLN were dissolved in physiological saline for injections. Chemicals were used without further purifications and distilled water was used

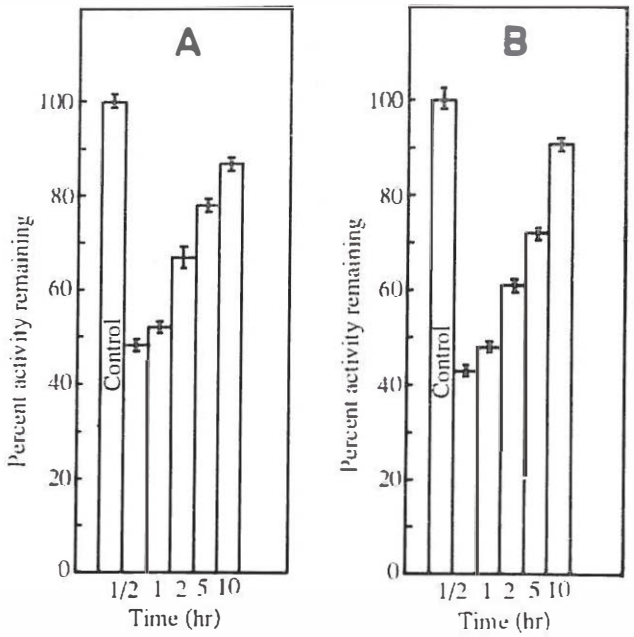

Fig. 1. Effect of peripheral injection of CCK-8(A) and/or CLN(B), 50 μg of each of per kg body weight, on GABA-T activity at different times after injection. N=6 for each determination. P<0.01 vs. control (student's t-test). The vertical bars represent ±S.D.

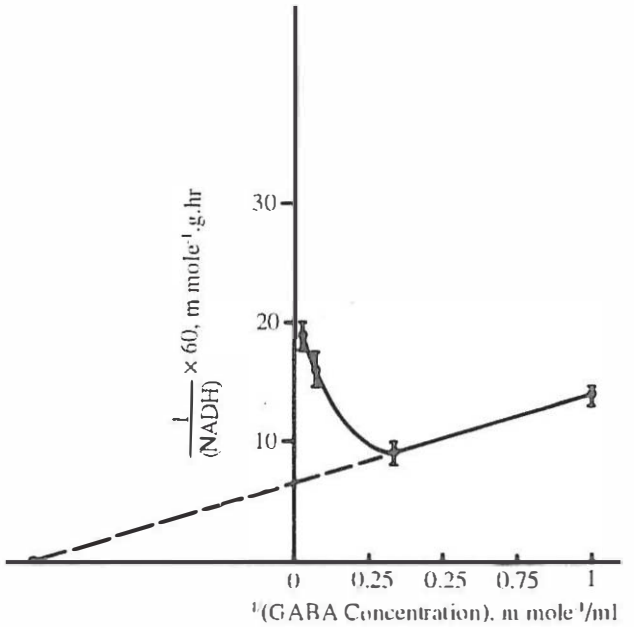

Fig. 2. GABA-T activity at different substrate concentrations. The Lineweaver-Burk plot of the NADH production by GABA-T at different GABA concentrations shows the inhibitory effect of the substrate on the enzyme activity at concentrations higher than 3.0 mmol/ml. N=6 for each determination. Vertical bars indicate ±S.D.

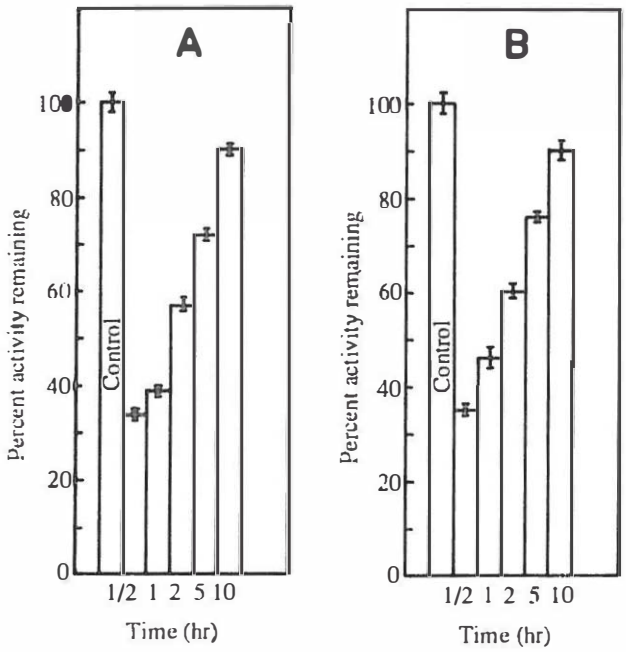

Fig. 3. Effect of peripheral injection of CCK-8(A) and/or CLN(B). 50 µg of each per kg body weight, on SSADH activity at different times after injection. N=6 for each determination. P<0.01 vs. control (student's t-test). The vertical bars represent ±S.D.

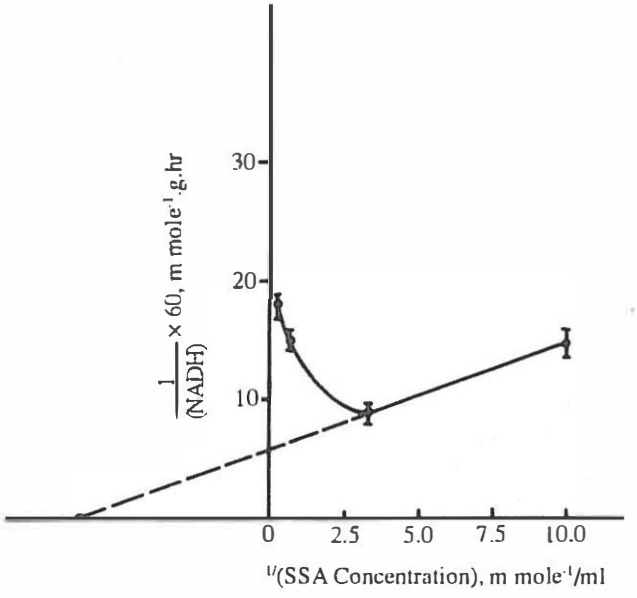

Fig. 4. SSADH activity at different substrate concentrations. The Lineweaver-Burk plot of the NADH production by SSADH at different SSA concentrations shows the inhibitory effect of the substrate on theenzyme activity at concentrations higher than 0.3 mmol /ml. N=6 for each determination. Vertical bars indicate ±S.D.

throughout this investigation.

Male NIH mice, weighing about 25-30 g purchased from Hesarak (Karaj, Iran) were kept in groups of 15 in cages in a room with 10-hr light cycle from 8 a.m. to 6. p.m. with free access to standard laboratory chow and water.

Methods

Tissue extract: Mice were injected with CCK-8 or CLN (50 µg/kg) intraperitoneally and the control group were injected with equal volume of physiological saline. The mice were killed by decapitation and their brain was removed from the skull in less than 1 minute. The hypothalami were removed from the brains on an ice chilled glass-plate, were quickly weighed, and finally were subjected to extraction: a 10% W/V homogenate was prepared by homogenizing the hypothalamus in a Teflon-glass homogenizer with an ice-cold solution containing 0.32 M sucrose and 4.5 mM 2-mercaptoethanol. A small portion of the homogenate was added to 3 volumes of ice-cold Triton medium (0.67% W/V Triton x-100, 50 mM Tris-HCl, pH 8.5, containing 4.5 mM 2-mercaptoethanol) and kept in ice water for 1 hr before use as described by Jung and Boer.[16,17]

Enzyme assays: For GABA-T assay a coupled enzyme system was used in which GABA was converted into SSA by transamination and subsequently oxidized to SA by the hypothalamic SSADH in the presence of NAD^+.[16,17] The incubation mixture contained: 50 mM Tris-HCl (pH 8.5), 3.0 mM GABA, 2.0 mM 2-oxoglutaric acid, 20 mM 2-mercaptoethanol, 1.1 mM NAD^+, and 0.2 ml of Triton-treated homogenate in a total volume of 1.125 ml. After a preincubation period of 30 min in the absence of GABA at 22°C, 0.1 ml of a 33.75 mM solution of GABA was added to the ice-cold incubation mixture and the reaction was started by keeping the reaction tubes at room temperature (22°C). The formation of NADH was measured spectrophotometrically at 340 nm as described by Jung.[16]

The SSADH activity of the hypothalamic homogenates was measured by converting the SSA to SA in the presence of NAD^+ according to the method described by Boer and Bruinvels.[17] The incubation mixture contained: 50 mM Tris-HCl (pH 8.5), 0.25 mM NAD^+, 0.3 mM SSA, 8.2 mM 2-mercaptoethanol, and 0.2 ml of Triton-treated homogenate in a total volume of 1.125 ml. After a preincubation period of 30 min at 22°C in the absence of SSA, 0.1 ml of 3.375 mM solution of SSA was added to the ice-cold incubation mixture and the reaction was started by keeping the reaction tubes at 22°C. The reaction was stopped after the appropriate incubation interval by placing the reaction tubes in ice water. The NADH production was measured by the method described for GABA-T assay.

TABLE I. The combined effects of CCK-8 and CLN on GABA-T activity in mouse hypothalamus

Percent GABA-T activity remaining after injection of CCK-8 (0.047 mmol/kg) and/or CLN (0.037 mmol/kg); observed values		Percent GABA-T activity remaining after injection of CCK-8 (0.024 mmol/kg) plus CLN (0.019 mmol/kg); observed values	Percent GABA-T activity remaining after injection of CCK-8 (0.024 mmol/kg) plus CLN (0.019 mmol/kg); calculated values
CCK-8	CLN	48.58±0.01	50.27±0.00
52.15±0.011	48.37±0.1		

The values represent mean ±S.D.% of control (control=100%).
P<0.01 vs. control (student's t-test). N=6 for each determination.

RESULTS

The acute effect of CLN and CCK-8 on GABA-T activity

The effect of CCK-8 and CLN on GABA-T activity is shown in Fig. 1A and Fig. 1B, respectively. As shown, both peptides caused a marked inhibition, about 55%, in GABA-T activity at the first half hour after peripheral injection of mice. However, as is evident from both parts of Fig. 1, the activity of the enzyme was gradually restored to about 90% of that of the control groups in 10 hours after peptide administration. The GABA-T activity was measured in all cases at 3.0 mM concentration of the substrate (GABA) which is, as shown in Fig, 2, in the non-inhibitory zone of the substrate concentration.

The acute effect of CCK-8 and CLN on SSADH activity

The peripheral injection of CCK-8 and/or CLN at a dose of 50 µg/kg body weight inhibited the SSADH activity by about 65 percent in the first 30 minutes after injection, as shown in Fig. 3. As with the GABA-T, almost full activity of SSADH was restored in 10 hours after injection. The activity of SSADH was measured at 0.3 mM concentration of the substrate which is, as shown in Fig. 4, a non-inhibitory concentration for the enzyme.

Combined effect of CCK-8 and CLN on GABA-T and SSADH activities

Table I shows the combined effect of CCK-8 and CLN on GABA-T activity in mouse brain at 3.0 mM concentration of GABA. The combined injected dose of CCK-8 and CLN (0.024 and 0.019 mmole/kg, respectively) inhibited the activity of GABA-T by 51 percent. The corresponding value calculated from the observed values, which were obtained from independent injection of CCK-8 and/or CLN showed 50 percent inhibition of the enzyme activity. Similarly, using a combined dose of CCK-8 and CLN (0.024 and 0.019 mmol/kg, respectively) at 0.3 mM concentration of SSA, the activity of SSADH was inhibited by 59 percent, compared to the calculated values of 57 percent, as shown in Table II.

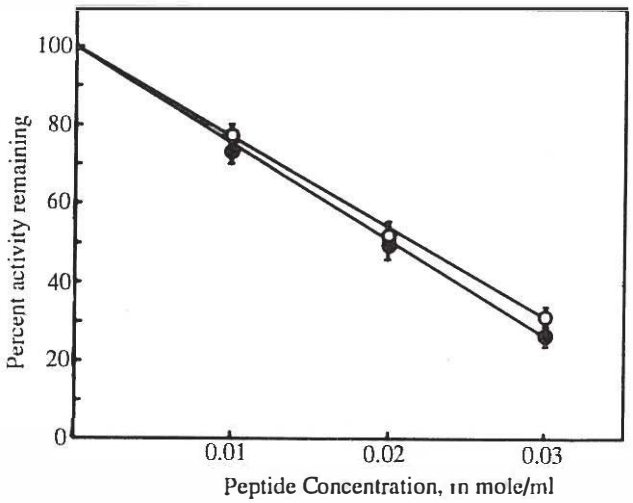

Fig. 5. The in vitro effect of CCK-8 (○-○) and /or CLN (●-●) on the GABA-T activity at different concentrations of each peptide. The substrate and the enzyme concentrations have been kept constant throughout the measurements at 3.0 and 8.15 mmol/ml, respectively. For experimental details see the experimental section.

In vitro effect of CCK-8 and/or CLN on GABA-T and SSADH activities

As shown in Fig. 5, both peptides could interact with the GABA-T and caused depression of its activity almost to the same extent. Similar observations were made concerning the SSADH activity in the presence of different concentrations of CCK-8 and/or CLN (Fig. 6). However, the full activity of both enzymes was restored after 30 hours of dialysis against Tris-HCl (50 mM, pH=8.5) buffer as shown in Table III.

DISCUSSION

The results obtained from *in vivo* and *in vitro* investigations showed that there is reversible inhibition of

TABLE II. The combined effects of CCK-8 and CLN on SSADH activity in mouse hypothalamus

Percent SSADH activity remaining after injection of CCK-8 (0.047 mmol/kg) and/or CLN (0.037 mmol/kg); observed values		Percent SSADH activity remaining after injection of CCK-8 (0.024 mmol/kg) plus CLN (0.019 mmol/kg); observed values	Percent SSADH activity remaining after injection of CCK-8 (0.024 mmol/kg) plus CLN (0.019 mmol/kg); calculatd values
CCK-8	CLN	40.53±0.002	42.47±0.00
38.76±0.002	46.17±0.02		

The values represent mean ±S.D.% of control (control=100%).
P<0.01 vs. control (student's t-test). N=6 for each determination.

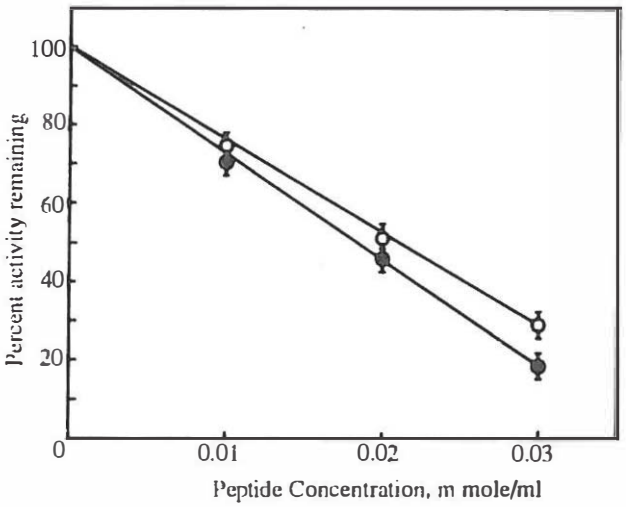

Fig. 6. The in vitro effect of CCK-8 (○-○) and/or CLN (●-●) on the SSADH activity at different concentrations of each peptide. The substrate and the enzyme concentrations have been kept constant throughout the measurements at 0.3 and 8.15 mmol/ml, respectively. For experimental details see the experimental section.

GABA-T and SSADH activity by CCK-8 and CLN. These observations indicate that there is an interaction between CCK-8 and/or CLN and the GABAergic system in the mouse brain. The present findings are in agreement with Nagahama's finding, which states that CCK-8 and/or CLN affect the GABA content and accumulation induced by AOAA treatment in the mouse striatum, hypothalamus, and frontal cortex.[13] In addition, it has been reported that CLN and CCK-8 enhance GABA release from rat cerebral cortex slices *in vitro*.[11] Other reports concerning the physiological effects of CLN and CCK-8 on monoaminergic, cholinergic, and GABAergic systems can be seen in the references.[11-13, 18,19]

Despite the accumulated data in the literature concerning the behavioral and physiological changes induced in the test animals by CCK-8 and/or CLN administration, the exact mechanism of action and the exact site(s) of action of these two peptides in the central nervous system have not been reported.

In the present study, it was indicated that there is direct interaction between CCK-8 and CLN on two of the catabolizing enzymes of GABA, namely, GABA-T and SSADH (Figs. 5 and 6). Based on the data shown in Figs. 1 and 2, similar effects have been observed *in vivo* concerning the acute inhibitory role of CCK-8 and CLN on GABA-T and SSADH. Inhibition of GABA-T and SSADH activity probably explains the increased GABA content in the mouse hypothalamus after peripheral injection of CCK-8 and CLN as reported by Nagahama.[15] In this case, it is vital to investigate the side effects(s) of higher GABA content on the whole enzyme systems of GABA metabolism, mainly on GAD. It is also necessary to investigate the acute and long lasting direct effect(s), if any, of CCK-8 and CLN on GAD in the central nervous system of mice. These investigations are in progress and will be published soon.

The combined effect of CCK-8 and CLN on GABA-T and SSADH activity compared to the independent and individual injection of CCK-8 and/or CLN, at the same molecular dose, as shown in Tables I and II, probably indicates that CCK-8 and CLN compete for the same site on the enzyme structure. The other alternative is the presence of two independent interaction sites on the enzyme with the same final effect on the enzyme activity. Further investigations are required to clarify these points.

In conclusion, the present data indicate that peripheral injection of CCK-8 and/or CLN at a dose level of 50 µg/kg body weight has acute inhibitory effects on GABA-T and SSADH, two of the catabolizing enzymes of GABA. The inhibitory effect is most probably due to the direct interaction between the enzymes and the peptides at the molecular level.

TABLE III. Effect of dialysis on the re-establishment of GABA-T and SSADH activity. For experimental details see the experimental section.

Sample		Percent Enzyme Activity Remaining	
		GABA-T	SSADH
before dialysis	Control	100±0.018	100±0.007
	CCK-8	25.28±0.007	29.06±0.008
	CLN	23.27±0.009	19.32±0.015
after dialysis	Control	95.08±0.013	98.10±0.006
	CCK-8	89.49±0.006	95.49±0.018
	CLN	91.50±0.015	96.85±0.01

The values represent mean±S.D.% of the control (control=100%). N=6 for each determination and $P<0.01$ vs. control (student's t-test)

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