fragments, E-T-1 and E-T-2, were separated by paper electrophoresis at pH 3.5. Their amino acid compositions are shown in the Table. E-T-2 was a dipeptide containing 1 mole of glutamic acid and 1 mole of lysine. Carboxypeptidase B digested E-T-2 to free lysine and pyroglutamic acid, which were identified by paper electrophoresis at pH 3.5. From this, the amino acid sequence of E-T-2 was deduced to be Pyr-Lys.

The C-terminal amino acid of E-T-1 was found by hydrazinolysis to be proline. The amino terminal part of E-T-1 was confirmed by direct Edman degradation to be

Trp-Asp-Pro-Pro-Pro-Val-

E-T-1 was hydrolyzed with a protease from *Streptomyces griseus*, and from the hydrolysate free tryptophan, valine and 2 peptide fragments, E-T-1a and E-T-1b, were separated by paper chromatography (*n*-butanol : pyridine : acetic acid : water = 15:10:3:12). The N-terminal amino acid of E-T-1a was found by direct Edman degradation to be aspartic acid and that of E-T-1b was found by subtractive Edman degradation to be serine. Consideration of these results and the amino acid compositions of E-T-1a and E-T-1b (Table), led to the conclusion that the amino acid sequence of E-T-1a and E-T-1b must be Asp-Pro-Pro-Pro and Ser-Pro-Pro, respectively.

The full structure of the potentiator must therefore be Pyr-Lys-Trp-Asp-Pro-Pro-Pro-Val-Ser-Pro-Pro.

Another bradykinin-potentiating peptide which contains tryptophan has been isolated from the venom of *Bothrops jararaca* and found by FERREIRA et al.[3] to have the structure Pyr-Lys-Trp-Ala-Pro (BPF 5a). The N-terminal part of the potentiator E is similar to that of the peptide BPF 5a. Although BPF 5a had a strong potentiating activity on guinea-pig ileum[4], the potentiator E had a weak activity in the same experiment. As against this, however, the potentiator E had a potent bradykinin-potentiating activity on the rat uterus. 0.75 µmole of potentiator E had a twofold potentiating effect on the bradykinin action on the guinea-pig ileum and 0.015 µmole of potentiator E had the same effect on the rat uterus. The potentiators B and C had, however, potent bradykinin-potentiating activities on the guinea-pig ileum, and weak activities on the rat uterus. These results suggest that the mechanism of the biological activities of bradykinin-potentiating peptides should be made the subject of further studies.

Amino acid composition of potentiator E and its fragments

Amino acid	E	E-T-1	E-T-2	E-T-1a	E-T-1b
Trp	0.8[a] (1)	N.D. (1)	–	–	–
Lys	0.7 (1)	–	0.9 (1)	–	–
Asp	0.8 (1)	1.2 (1)	–	1.0 (1)	–
Ser	0.7 (1)	0.9 (1)	–	–	0.8 (1)
Glu	1.0 (1)	–	1.0 (1)	–	–
Pro	4.1 (5)	5.1 (5)	–	3.0 (3)	2.0 (2)
Val	1.1 (1)	1.0 (1)	–	–	–

N.D., not determined. [a] Determined spectrophotometrically by the method of GOODWIN and MORTON[5].

Zusammenfassung. Es wird über die Strukturaufklärung eines Bradykinin-potenzierenden Peptids aus dem Gift von *Agkistrodon halys blomhoffii* berichtet.

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The Effect of Guanidinosuccinic Acid on in-vitro Carbohydrate Metabolism

Glucose intolerance is well-documented in chronic uremia[1]. Although the early response of insulin to various stimuli may be blunted, the eventual attainment of normal to increased levels has suggested that insulin antagonism may play a role in this condition[1]. Guanidinosuccinic acid (GSA), undetectable in normal individuals (< 0.15 mg/100 ml) accumulates in the serum of patients with renal insufficiency (mean — 2.53 mg/100 ml)[2]. It has been postulated that the 'defect in cellular glucose uptake in uremia' is secondary to the presence of this compound[3]. Accordingly, the effect of GSA on the in vitro response of the rat diaphragm to glucose and insulin was studied.

Methods. The rat diaphragm assay for assessment of indulin antagonism has been described previously[4]. Briefly, it involves the serial incubation of paired hemidiaphragms, first in 2.0 ml of buffered glucose (2.0 mg/ml) alone and then in media containing added insulin (500 µU/ml). One of each pair of hemidiaphragms was exposed to GSA (0.03 mg/ml). Thus, basal and insulin-stimulated glucose uptakes in the presence and absence of GSA were obtained on the tissues from each animal which avoids the marked variation observed with hemidiaphragms from separate rats[1,4].

Results. The Table shows that GSA did not affect basal or insulin-stimulated glucose uptake of paired rat hemidiaphragms in vitro. The insulin effect (I–B) did not differ when the *t*-test for differences between paired observations was used to compare the data but was significantly increased ($p < 0.05$) in the presence of GSA if the *t*-test for differences between means was utilized.

Discussion. The data presented here do not support the hypothesis that GSA accumulation in uremia is

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responsible for glucose intolerance. In addition, serum from patients before and after dialysis had the same effect on the in vitro activity of added insulin against rat hemidiaphragm in spite of the fact that intravenous glucose tolerance improved in every patient after treatment[1]. This speaks against a circulating insulin antagonist and is consistent with the negative effect of GSA on in vitro carbohydrate metabolism. Furthermore, improvement of glucose tolerance and recovery of sensitivity to injected insulin only after repeated dialyses[5] could not be explained by an effect of the readily dialyzable[2] compound GSA. Glucose intolerance in chronic renal failure would seem to be secondary to peripheral tissue insensitivity to the action of insulin[1,6] and/or a delayed pancreatic insulin response to glucose[6].

The effect of GSA (0.03 mg/ml) on the in-vitro response of paired rat hemidiaphragms[a] to glucose (2.0 mg/ml) and insulin (500 μU/ml)

	Basal		Insulin		I–B	
Mean	2.68[b]	2.31	5.94	6.27	3.26	3.96
± S.E.	0.22	0.16	0.22	0.27	0.18	0.28
GSA	—	+	—	+	—	+

[a] 10 animals. [b] μg/mg/h.

Zusammenfassung. Da Guanidinosuccinsäure in der Urämie akkumuliert, wurde angenommen, dass dieses Stoffwechselprodukt bei Niereninsuffizienz die bekannte Glukoseintoleranz verursachen könnte. In-vitro-Versuche am Rattenzwerchfell haben diese Hypothese jedoch nicht bestätigen können, da Guanidinosuccinsäure in der Konzentration von 3 mg/100 ml keinen Einfluss auf die basale oder insulinstimulierte Glucoseaufnahme durch dieses Gewebe zeigte.

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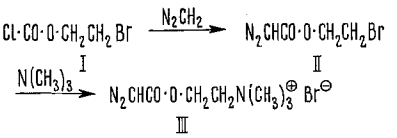
Diazoacetyl Choline Bromide

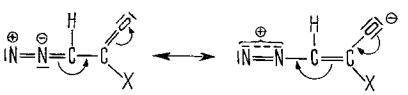
We have synthesized diazoacetyl choline bromide (III) in order to evaluate its potentialities as an affinity label for acetyl cholinesterase, choline acetyl transferase and, especially, acetyl choline receptor molecules. Diazo analogs of enzyme substrates have been shown to react with sterically favorable groups of the binding site of the enzyme either in the dark (in the presence[1–3] or absence[3–5] of cupric ions) or during photolysis[6–8]. In the dark reaction, the diazo compounds presumably attack carboxyl groups of the enzyme. Photolysis produces carbenes which may be reactive enough to insert themselves even into carbon hydrogen bonds[9]. Diazo ketones and diazo esters may, in addition, undergo a WOLFF rearrangement[10–13] before producing covalent bonds with functional groups of the enzyme.

Synthesis proceeded in a straightforward manner according to the following scheme:



2-Bromoethyl chloroformate (I, product of FLUKA Ltd., Buchs, Switzerland) was treated with diazomethane to yield 2-bromoethyl diazoacetate (II). A small amount of a contaminant, presumably 2-bromoethyl chloroacetate, was removed by chromatography. Pure II was then treated with trimethylamine in toluene to yield essentially pure, crystalline diazoacetyl choline bromide (III)[14].

Hydrolysis of diazoacetyl choline bromide (III) by the enzyme acetyl cholinesterase from Electrophorus electricus at pH = 7 and 25 °C proceeds approximately 1.6×10^4 times more slowly than that of acetyl choline iodide. This phenomenon might be caused by the topochemical differences between the two molecules, but it might also be due to the inactivation of the ester linkage in esters of diazoacetic acid. It was found in our laboratory that *p*-nitrophenyl diazoacetate[8] does not behave like an active ester[15], but reacts very much more slowly with amines. An electronic mechanism is probably operative which makes it more difficult for the carbonyl C-atom to assume a partial positive charge during attack by a nucleophile. A plausible electron distribution is delineated in the following electromeric formulae:



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