

Haematocrit and platelet counts were performed in 5 animals, during the control period and during intoxication at intervals of 5 days. In 3 animals the bleeding time was controlled by inducing a superficial wound in an ear. At the end of intoxication, which was prolonged for 30–45 days, all the animals were killed with an i.v. injection of pentobarbital and submitted to necropsy.

Results. The only symptom which appeared following the administration of urea was a mild drowsiness with reduction in the spontaneous movements. The EEG recorded during intoxication revealed minimal changes similar to those induced by tranquillizing drugs.

No other abnormalities were observed. Dogs did not suffer from g.i. disturbances but continued feeding regularly and, at the end of intoxication, their weight had not changed. Diuresis was, of course, increased due to the diuretic action of the urea, and consequently the ingestion of water also increased. Haematocrit, platelet counts and bleeding time did not change.

Necropsy was completely negative, and histological examination did not reveal any significant abnormality in the liver, heart, kidney, stomach and duodenum.

Conclusions. The conclusion to be drawn from these results is that urea does not induce any severe toxic symptom in dogs, not even when its plasma concentra-

tions were higher than those found in severe chronic uremics. Of all the typical symptoms of human uremia, only drowsiness occurred, and even this disturbance disappeared as soon as administration of urea was interrupted. It seems safe to conclude that urea cannot be considered an important uremic toxin⁸.

Riassunto. Le concentrazioni plasmatiche dell'urea di 12 cani normali venivano mantenute fra 250 e 650 mg/100 ml per 25–30 giorni mediante iniezioni ravvicinate di una soluzione al 10%. Nessun sintomo veniva osservato ad eccezione di un lieve torpore e l'esame necroscopico era del tutto negativo.

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Hemmung der Kininbildung im Blut durch *p*-Amidinophenylbrenztraubensäure

In den vorangegangenen Untersuchungen über die Hemmung der kininbildenden Fermente (Kallikreine) durch Benzamidinderivate konnte gezeigt werden, dass die als kompetitive Hemmstoffe des Trypsins bekannten synthetischen Inhibitoren eine starke Hemmung des Serulkallikreins bewirken, während das Pankreaskallikrein bei gleicher Inhibitorosis nicht gehemmt wurde^{1,2}. Als stärkster Hemmstoff des Serulkallikreins hatte sich die von GERATZ³ als Trypsinhemmstoff beschriebene *p*-Amidinophenylbrenztraubensäure (APPA) erwiesen. Diese in vitro erhobenen Befunde sind von besonderem Interesse, da die pharmakologische Beeinflussung des Kallikrein-Kinin-Systems an Bedeutung gewinnt und zur Klärung der physiologischen und pathologischen Rolle dieser proteolytischen Reaktionskette beitragen kann. Wir haben daher geprüft, ob sich mit Hilfe von APPA auch die Kininbildung im Blut beeinflussen lässt.

Es wurde zunächst der Einfluss von APPA auf die durch Pankreaskallikrein (Präparat aus Schweinepankreas mit einer Aktivität von 525 KE/mg) und Serulkallikrein (nach dem Verfahren von HABERMANN⁴ aus Schweineserum isoliertes Präparat; Aktivität entspricht 1 KE/mg) bewirkte Kininbildung im Serum untersucht. Die Bestimmung der gebildeten Kininmenge erfolgte durch Messung ihrer kontraktionsauslösenden Wirkung am isolierten Meerschweinchenileum (Versuchsbeispiel Figur 1). Demnach wird die Kininbildung durch Serulkallikrein in Abhängigkeit von der APPA-Konzentration gehemmt ($I_{50} = 6 \times 10^{-6} M$). Die Kininfreisetzung durch Pankreaskallikrein wird dagegen in Übereinstimmung mit

Untersuchungen anhand der Hydrolyse von synthetischen Substraten erst bei sehr viel höheren APPA-Konzentrationen beeinflusst ($I_{50} > 10^{-3} M$).

Zum Nachweis der Antikallikrein-Wirkung in vivo wurde versucht, die durch Injektion von Serum- oder

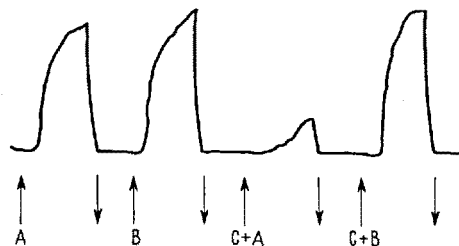


Fig. 1. Einfluss von 4-Amidinophenylbrenztraubensäure (APPA) auf die Kininbildung im Serum durch Serulkallikrein (SK) und Pankreaskallikrein (PK). Messung der Kininwirkung am isolierten Meerschweinchenileum in 10 ml Tyrodelösung mit 0,5 ml Humanerum. A) Zugabe von 0,5 Einheiten SK. B) Zugabe von 0,5 Einheiten PK. C) Zugabe von 0,1 μ Mol APPA.

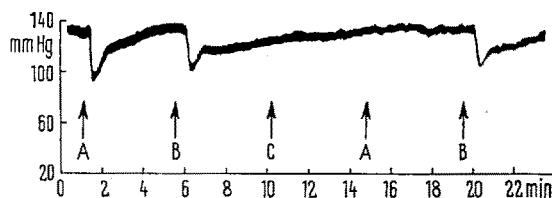


Fig. 2. Wirkung von APPA auf den blutdrucksenkenden Effekt von Serulkallikrein (SK) und Pankreaskallikrein (PK) beim Kaninchen (Blutdruckmessung in der A. carotis communis). A) i.v. Injektion von SK 3 KE/kg. B) i.v. Injektion von PK 2 KE/kg. C) i.v. Injektion von APPA 1,0 mg/kg.

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Pankreaskallikrein bewirkte Blutdrucksenkung durch vorherige Gabe des Hemmstoffes zu verhindern. Die Untersuchungen wurden an insgesamt 20 2,8–3,2 kg schweren Kaninchen in Urethannarkose (1,5 g/kg i.p.) durchgeführt. Die Versuchsanordnung wurde so getroffen, dass zunächst die blutdrucksenkende Wirkung von steigenden Mengen Serum- bzw. Pankreaskallikrein gemessen wurde. Anschliessend wurde APPA appliziert und danach versucht, wiederum eine Blutdrucksenkung durch Injektion von Serum- bzw. Pankreaskallikrein auszulösen. Wie aus dem in Figur 2 dargestellten Versuchsbeispiel ersichtlich ist, wird durch Injektion von 1 mg APPA/kg die durch Serumkallikrein bewirkte Hypotension verhindert. Entsprechend der Elimination von APPA (Halbwertszeit = 104 min)⁵ hält die Antikallikreinwirkung bei der Gabe von 1 mg/kg bis zu 30 min und bei der Gabe von 2,5 mg/kg bis zu 2 $\frac{1}{2}$ Stunden nach der Injektion an. Danach verursachen auch Injektionen von

Serumkallikrein wieder blutdrucksenkende Effekte. Die blutdrucksenkende Wirkung von Pankreaskallikrein wird bei Gabe der gleichen APPA-Dosis nicht signifikant beeinflusst.

Summary. The serum kallikrein-induced kinin liberation in the blood is inhibited by amidino phenyl pyruvic acid (APPA) in vitro and in vivo.

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Synaptic Connections made by two Serotonin-Containing Neurons in the Snail (*Helix pomatia*) Brain

There is a giant serotonin-containing neuron in each cerebral ganglion of the snail *Helix pomatia*¹. This report describes the results of experiments made to locate different neurons innervated by these cells and to determine the routes of the connections. Efforts to find such cells have been centred on the buccal ganglia because previous dye injection and electrophysiological experiments^{2,3} have shown that axons from the giant serotonin cells (GSCs) pass into the cerebro-buccal connectives.

Materials and methods. Electrophysiological methods were used. The circum-oesophageal nerve ring and the buccal ganglia were removed from live *H. pomatia* and pinned to a plastic sheet at the base of a small, 0.7 ml, chamber perfused with saline⁴. A double barrel glass microelectrode was inserted into one of the GSC. Similar electrodes were used to impale selected cells in each buccal ganglion. Input leads from the electrodes were fed through cathode followers to a dual beam oscilloscope or a pen recorder.

Results and discussion. Three large neurons can be seen at the lateral borders of each buccal ganglion. These cells have been called anterior, middle and posterior buccal cells. They are diagrammatically represented in Figure 1. It has been found that each of these cells receives an input from each GSC. In the case of the posterior and middle cells the input is almost certainly mono-synaptic. The input onto the anterior cells is different from that of the other cells and may involve a less frequently observed mechanism of synaptic transmission.

Spike firing in either GSC resulted in the appearance of small depolarizing potentials in both ipsilateral and contralateral posterior and middle buccal cells. The amplitude of such potentials was facilitated with repetitive firing of the GSC and there was summation which lead to spike firing. When the appropriate buccal cells were artificially hyperpolarized, the amplitude of the small potentials was increased indicating that the potentials are excitatory postsynaptic potentials (EPSPs).

The latency between GSC spike and the appearance of the EPSP in the buccal cells was constant at about 100 msec. No consistent difference in latency was observed between ipsi- and contralateral connections. There was a one to one relationship between GSC spike and buccal cell EPSP even after several hundred GSCS

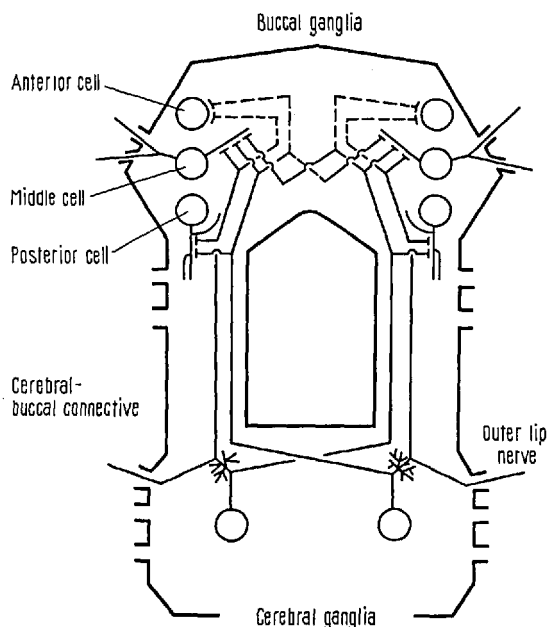


Fig. 1. Diagram showing the two GSCs in the cerebral ganglia, their processes, and the neurons with which they form synaptic contacts. The posteriorly placed cell and the middle cell in each buccal ganglion are most probably directly innervated by each GSC; the precise nature of the connections between the GSC and the anteriorly placed cell in each ganglion remains to be determined. The processes from the different cells were traced either by dye injection or by electrophysiological methods (see text). The exact points of contact between the cells is not known, but the synapses are probably axo-axonic since most if not all synapses in gastropod ganglia are of this type⁶. Note the position of the arborization of each GSC and the double innervation of the 'middle cell' in each buccal ganglion by the appropriate ipsilateral GSC.

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