Effect of calcium and temperature on spontaneous and 48/80 induced histamine release from pig lung

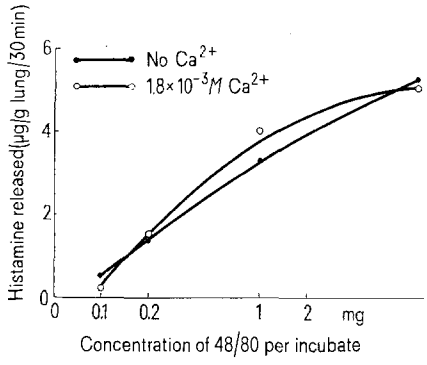
	Histamine released (μg/g lung/30 min) 37 °C		4 °C	
$[Ca^{2+}]$	Spontaneous	48/80 (2 mg)[a]	Spontaneous	48/80 (2 mg)[a]
0	1.21 ± 0.23 (4)	5.29 ± 0.57 (4)		
1.8×10^{-3}	1.11 ± 0.16 (3)	5.12 ± 0.67 (3)	0.83 ± 0.09 (3)	2.11 ± 0.28 (3)
1.8×10^{-2}	1.16 ± 0.29 (3)	2.79 ± 0.51 (3)		

Figures are means ± S.E.M. Figures in parentheses refer to number of experiments. [a]Corrected for spontaneous release. Average total histamine was 28.52 (16.2–41.5) μg/g lung, $n = 5$.

SHORE et al.[10]. The data are expressed as μg histamine base released per g wet weight of chopped lung.

Results and discussion. Compound 48/80 released histamine from chopped pig lung in a dose dependent manner both in the presence and absence of calcium (Figure). There was no difference in the amount of histamine released either spontaneously or with 48/80 in the absence or in the presence of 1.8×10^{-3} *M* calcium (Table). When the calcium concentration was increased to 1.8×10^{-2} *M* the amount of histamine released spontaneously and with 48/80 was inhibited (Table). The amount of histamine released in the presence of 1.8×10^{-3} *M* calcium was reduced when the incubation took place at 4 °C compared to that released at 37 °C (Table).

Histamine release from chopped lung by compound 48/80 differs from anaphylactic histamine release from this tissue in that the release has a longer time course[8], calcium is not required, and the release is not completely abolished by low temperatures. The concentration of 48/80 required for the minimum detectable amount of histamine release from chopped lung from several species is 2 to 3 orders of magnitude higher than that required for histamine release from rat peritoneal mast cells[8,11]. In contrast, the amount of antigen required to release histamine from sensitized mast cells and chopped guinea-pig lung is of the same order of magnitude[2,5]. It is thus possible that histamine release occurs by 2 mechanisms. 1. Release by low concentrations of 48/80 and in anaphylaxis is by a calcium dependent mechanism. 2. Release by high concentrations of 48/80 as required for chopped lung is by a different calcium independent mechanism. Thus histamine release from chopped lung by compound 48/80 may not be a useful model for the study of anaphylactic histamine release from this tissue.



Histamine release by various concentrations of 48/80 in the presence and absence of calcium. The data are corrected for spontaneous release.

Zusammenfassung. Es zeigt sich, dass die anaphylaktische Histaminausschüttung Calcium benötigt und bei niedriger Temperatur vollkommen gehemmt wird. Bei Zugabe der Substanz 48/80 erweist sich die dadurch hervorgerufene Histaminausschüttung als dosisabhängig und benötigt kein extrazelluläres Calcium. Die Reaktion wird bei niedriger Temperatur (4 °C) nur teilweise gehemmt. Sie kann daher nicht als zweckmässiges Modell für das Studium der anaphylaktischen Histaminausschüttung der Lunge angesehen werden.

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5-HT Hyperpolarization of Bullfrog Sympathetic Ganglion Cell Membrane

It is well known that the slow inhibitory postsynaptic potentials (slow IPSP) of sympathetic ganglion cells are produced by an activation of preganglionic nerve fibres, and that these potentials can be produced in the presence of nicotine or D-tubocurarine but are blocked by atropine[1,2]. A hyperpolarization of ganglion cells is produced by a direct application of acetylcholine (ACh) to ganglia, and the nature of this ACh hyperpolarization, which could be eliminated in a Ca-deficient Ringer's solution containing Mg, is essentially similar to that of the slow IPSP[2]. Thus, the slow IPSP does not seem to be produced by a direct action of ACh but rather by the action of some transmitter released from intermediating

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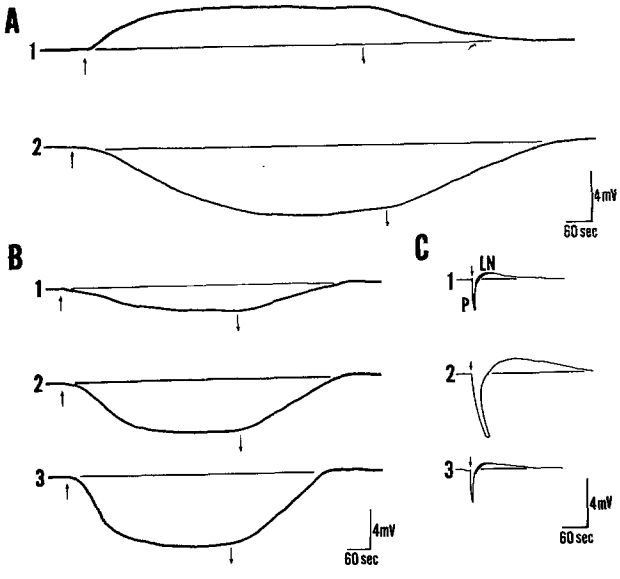
interneurones, which are activated by ACh, in ganglia. In the case of amphibian sympathetic ganglia, a number of experimental results suggest that adrenaline (Ad) may be the transmitter which is directly responsible for the generation of slow IPSP or ACh hyperpolarization[2]. It must be noted, however, that no conclusive experimental evidence has yet been provided to prove that the transmitter for the slow IPSP is actually Ad.

It has been found in the present experiment that 5-hydroxytryptamine (5-HT) is also able to hyperpolarize the bullfrog sympathetic ganglion cell membrane. Such a 5-HT hyperpolarization seems to be produced by a direct action of 5-HT on the ganglion cell membrane, and the nature of this hyperpolarization is essentially similar to that of the slow IPSP or ACh hyperpolarization.

Materials and methods. Paravertebral sympathetic ganglion chains of bullfrogs (*Rana catesbiana*) were used throughout in the present experiment. Membrane potential changes of ganglion cells were recorded by use of sucrose-gap method[3]. Experimental solutions and their ionic compositions (m*M* per 1000 cm^3 H_2O) are as follows: Ringer's solutions (112 m*M* NaCl, 2 m*M* KCl, 1.8 m*M* $CaCl_2$, 2 m*M* $NaHCO_3$), Ca-deficient Ringer's solution containing Mg (112 m*M* NaCl, 2 m*M* KCl, 0.18 m*M* $CaCl_2$, 6 m*M* $MgCl_2$) and Na-free lithium solution (114 m*M* LiCl, 2 m*M* $KHCO_3$ and 1.8 m*M* $CaCl_2$). Drugs used in the present experiment are as follows: 5:hydroxytryptamine creatinine sulfate (Wako), ouabain (Merk), nicotine sulfate (Katayama), D-tubocurarine chloride (Merk) and atropine sulfate (Wako).

Results and discussion. A sympathetic ganglion was continuously perfused with the Ringer's solution. When 5-HT ($1–10^{-2}$ m*M*) was added to the Ringer's solution, the ganglion cell membrane was depolarized (Figure A-1).



Effects of 1 m*M* 5-HT on the membrane potential of bullfrog sympathetic ganglion cells. A) 5-HT depolarization (1) and 5-HT hyperpolarization (2), recorded from a preparation before and 40 min after an application of 0.12 m*M* nicotine respectively. B) Effects of conditioning depolarization (1) and conditioning hyperpolarization (3) on a 5-HT hyperpolarization (2). C) Effects of 5-HT on the slow IPSP (P) and slow EPSP (LN) produced by a train of repetitive preganglionic nerve stimulation (30/sec for 4 sec) in a nicotinized ganglion. Records 1 and 2 were recorded before and 3 min after an application of 5-HT respectively, and record 3 was 10 min after withdrawal of 5-HT. Arrows in A and B indicate duration of 5-HT applications and those in C represent beginning of nerve stimulation.

The depolarization was sustained during an application of 5-HT (20–30 min) and it was restored upon its withdrawal. Although the amplitude of 5-HT depolarization varied according to individual preparations, it was consistently observed, even when cholinergic transmissions in ganglia[2] were completely blocked under the effects of D-tubocurarine and/or atropine.

An interesting finding was the experimental fact that the ganglion cell membrane was hyperpolarized instead of being depolarized by the action of 5-HT ($1–10^{-2}$ m*M*) in the presence of nicotine. The cholinergic (nicotinic) transmission in ganglia was completely blocked when nicotine (0.12 m*M*) was applied for more than 30 min to a preparation[4]. Under such an experimental condition, ganglion cell membrane was found to be markedly hyperpolarized by the action of 5-HT (1 m*M*), as seen in Figure A-2. This 5-HT hyperpolarization tended to fall after it reached a maximum amplitude and it was rapidly restored upon withdrawal of 5-HT (Figure A-2). The 5-HT hyperpolarization was also observed when atropine (0.1 m*M*) was applied together with nicotine before 5-HT was applied.

Both the 5-HT depolarization (without nicotine) and 5-HT hyperpolarization (with nicotine) remained unchanged, even when the Ca-deficient Ringer's solution containing Mg was used instead of the Ringer's solution.

A comparison between the nature of 5-HT hyperpolarization and that of slow IPSP is very interesting. According to the present experiment, the nature of the 5-HT was essentially similar to that of the slow IPSP[3,5,6]. Namely, 1. the amplitude of the 5-HT hyperpolarization was markedly augmented under a conditioning hyperpolarization, whereas it was depressed under a conditioning depolarization of ganglion cell membrane (Figure B), 2. the 5-HT hyperpolarization was sensitive to ouabain, being almost completely depressed by it in a concentration of 10^{-2} m*M*, and 3. the 5-HT hyperpolarization was eliminated in the Na-free lithium solution.

The slow IPSP of sympathetic ganglion cells can be recorded by applying repetitive preganglionic nerve stimulation (30/sec for 4 sec) in the presence of nicotine[4]. A remarkable augmentation of the slow IPSP was consistently observed during a development of the 5-HT hyperpolarization (Figure C). Such a large augmentation could not be observed when ganglion cell membrane was hyperpolarized by applying a conditioning anodal current[3] in the absence of 5-HT.

As has been shown in the present experiment, 5-HT causes the depolarization of ganglion cell membrane in the Ringer's solution containing D-tubocurarine and/or atropine, while it causes the hyperpolarization in the presence of nicotine. Since the 5-HT hyperpolarization can be produced even when atropine is added, it is clear that ACh, which might be released from preganglionic nerve terminals by the action of 5-HT, is not directly responsible for the initiation of both 5-HT depolarization and 5-HT hyperpolarozation. Furthermore, the experimental fact that both the 5-HT depolarization and 5-HT hyperpolarization can be produced in the Ca-deficient Ringers's solution containing Mg, suggests that these potential changes are produced by a direct action of 5-HT on the ganglion cell membrane. Thus, a most reasonable assumption based on the present experimental

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results may be as follows: bullfrog sympathetic ganglion cells possess two different kinds of receptors which are sensitive to 5-HT, and one receptor which is responsible for the 5-HT depolarization can be blocked by nicotine and another receptor which is responsible for the 5-HT hyperpolarization is resistant to nicotine. Presumably, the 5-HT hyperpolarization is disclosed when the 5-HT depolarization is completely blocked by the action of nicotine.

According to the present experiment, the 5-HT hyperpolarization is augmented during a conditioning hyperpolarization, and it is sensitive to ouabain and eliminated in the Na-free lithium solution. The nature of the 5-HT hyperpolarieation is essentially similar to those of the slow IPSP, ACh hyperpolarization and Ad hyperpolarization[2,3,5-7]. Thus it is feasible that the 5-HT hyperpolarization may be generated by an activation of electrogenic sodium-pump, as has been suggested in the cases of the slow IPSP, ACh hyperpolarization and Ad hyperpolarization[3,6,7].

The possibility that 5-HT might be the transmitter which is directly responsible for a generation of the slow IPSP is doubtful, since the slow IPSP is markedly augmented in the presence of 5-HT. It is known that the size of the slow IPSP is augmented when the ganglion cell membrane is hyperpolarized by conditioning anodal current[3]. The observed augmentation of the slow IPSP, however, was so powerful that it is difficult to explain this augmentation simply as a result of the 5-HT hyperpolarization. Whatever the mechanism underlying the slow IPSP may be, 5-HT appears to accelerate the process of the generation of the slow IPSP. Thus, although 5-HT may not be a transmitter, further analysis of the mode of action of 5-HT to ganglion cell membrane may provide some key to understand the mode of action of an actual transmitter which is responsible for the slow IPSP.

Zusammenfassung. Die Membran der sympathischen Ganglienzelle des Ochsenfrosches (*Rana catesbiana*) wird durch 5-HT depolarisiert; 5-HT erzeugt jedoch in Gegenwart von Nicotin eine Hyperpolarisation. Es scheint, dass sowohl die Depolarisation als auch die Hyperpolarisation von 5-HT durch direkte Wirkung auf die Zell-Membran zustande kommt. Die durch 5-HT erzeugte Hyperpolarisation zeigte ähnliche Eigenschaften wie das «slow IPSP» welches jedoch während der Entwicklung der 5-HT Hyperpolarisation stark zunimmt. Es wird vermutet, dass 5-HT wahrscheinlich nicht als Überträgerstoff für das «slow IPSP» in Frage kommt.

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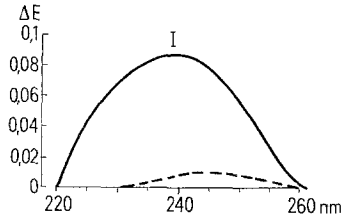
Silymarin verhindert die Lipidperoxidation bei der CCl_4-Leberschädigung

In einer vorausgegangenen Arbeit wurde mitgeteilt, dass einige aliphatische und insbesondere heterocyclische Mono- und Dithiolverbindungen sowohl die Zellschädigung als auch die Lipidperoxidation bei der CCl_4-Leberschädigung der Ratte unterbinden[1]. Wir beschreiben hier, Silymarin wirke nicht nur antihepatotoxisch in dem Sinne, dass es präventiv und kurativ die mit einem Zellschädigungsprozess verbundene Abgabe leberspezifischer Enzyme an das Blut unterbindet[2,3], sondern dass es auch die Lipidperoxidation hemmt. Inwieweit diese eine Rolle bei der CCl_4-Leberschädigung spielt, wurde früher diskutiert[2-5].

Die hier verwendete Versuchstechnik lehnt sich an solche des RECKNAGELschen Arbeitskreises an[6,7] und wurde bereits ausführlich beschrieben[1]. Wir verwendeten das uns von der Fa. Dr. Madaus & Co., Köln-Merheim, dankenswerterweise zur Verfügung gestellte Silymarin aus *Silybum marianum* (L.) Gaertn. als N-Methylglucamin-Salz.



Differenzspektren der mikrosomalen Lipide aus Rattenlebern (gemessen gegen Mikrosomenlipide aus Lebern unbehandelter Tiere). —, Kontrollen ohne Silymarin; ---, Versuche mit Silymarin. 60 min vor dem Töten einmalige i.p. Verabreichung von 140,5 mg Silymarin-N-methylglucamin-Salz gelöst in Dimethyl-sulfoxid: Wasser 1:1, pH 7,4 (Kontrollen: Applikation gleicher Mengen Solvens), 30 min später CCl_4-Intoxikation: 0,25 ml CCl_4, 1:1 in Paraffinöl/Tier per os; dreitägige Vorbehandlung mit je 80 mg Na-Barbital/Tier i.p.

Das Kurvenbild zeigt anhand der Differenzspektren, dass das Silymarinderivat am wirksamsten von allen seither geprüften Verbindungen, auch der eingangs erwähnten Thiole, die mikrosomale Lipidperoxidation hemmt.

Summary. Silymarine-N-methylglucamine-salt has a pronounced high antioxidative function against microsomal lipid peroxidation, associated with liver injury by CCl_4.

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