Die Leucinaminopeptidaseaktivität des Rattenharnes nach Verabreichung einer mastzellerschöpfenden Substanz

In Mastzellen des Menschen und verschiedener Laboratoriumstiere wurde histochemisch eine Leucinaminopeptidaseaktivität (LAP) nachgewiesen[1]. Klinische und experimentelle Untersuchungen sprechen für eine Freisetzung von LAP bei der Mastzelldegranulierung. So erfolgt nach Schädigung von Mastzellen durch hypotone Lösungen ein Austritt von LAP in das Suspensionsmedium[2]. Nach Schlangenbiss ist die LAP-Aktivität des Serums erhöht[3], was mit der mastzelldegranulierenden Wirkung der Schlangengifte in Zusammenhang stehen könnte[4]. Die Bedeutung der LAP-Freisetzung ist bisher noch ungeklärt. Möglicherweise liefert die LAP durch Abspaltung von Histidin aus Proteinen das Substrat für die Histidindecarboxylase der Mastzellen[5]; auf diese Weise könnte die LAP an der Histaminbildung beteiligt sein.

Der Harn von Mensch und Ratte enthält eine LAP-Aktivität[6–8]. Da bisher noch keine Untersuchungen über Beziehungen zwischen LAP-Aktivität des Harnes und Mastzelldegranulierung vorliegen, wurde die LAP-Ausscheidung nach Verabreichung der klassischen mastzellerschöpfenden Substanz 48/80 bestimmt[9].

Methodik. Um vergleichbare Harnmengen zu erhalten, wurde an männliche Albinoratten täglich 10 ml Wasser mit der Schlundsonde verabreicht. Die LAP-Aktivität des 24 h Harnes wurde in folgender Weise bestimmt: 4 mg L-Leucyl-β-Naphthylamin-HCl in 2,0 ml 0,05 m Phosphatpuffer (pH 7,2) wurden mit 0,05 ml frischem zentrifugiertem Harn 1 h bei 37° inkubiert. Das durch die Wirkung der LAP und bestimmter Endopeptidasen[10] gebildete β-Naphthylamin wurde nach Kupplung mit Echtrot 3 GL photometrisch erfasst. Die LAP-Aktivität von 1 ml Harn,die unter den genannten Bedingungen in 1 h 1 μg β-Naphthylamin freisetzt, wurde als 1 LAP-Einheit definiert. Nach Ermittlung der Normalwerte (70 Ratten) erhielten 20 Tiere intraperitoneal je 0,5 mg Compound 48/80 in isotoner Kochsalzlösung.

Die Ergebnisse sind in der Tabelle zusammengestellt. Im 24-h-Harn ist nach Verabreichung von Compound 48/80 eine statistisch signifikante Erhöhung der LAP-Aktivität nachzuweisen, die mit der Mastzelldegranulierung in Zusammenhang stehen könnte. Ob eine Ausscheidung der freigesetzten Mastzellen-LAP vorliegt, oder ob die anaphylaktoide Schockreaktion an den Nierenepithelien die Freisetzung der LAP hervorruft wird zur Zeit geprüft. Vorläufige Untersuchungen ergaben, dass auch ein anaphylaktischer Schock bei der Ratte zu einem Anstieg der LAP-Aktivität des Harnes führt.

	LAP-Einheiten des Harnes	
	pro ml	in der 24-h-Menge
Normale Ratten (70 Tiere)	136 ± 84	1054 ± 609
Nach intraperitonealer Verabreichung von 0,5 mg Compound 48/80 (20 Tiere)	707 ± 541 $P < 0{,}0005$	6002 ± 1020 $P < 0{,}0005$

Summary. LAP activity was determined in rat urine under normal conditions and following mast cell depletion by compound 48/80. A statistically significant increase in urinary LAP activity is found after administration of compound 48/80. This increase is due to mast cell depletion and the resulting anaphylactoid reaction.

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[7] H. BERGMANN und G. F. KLOSTERMANN, Arch. klin. exp. Derm. *218*, 603 (1964).
[8] H. BERGMANN und F. TRUSS, Mediz. Welt *1964*, 1760.
[9] Compound 48/80 (Burroughs Wellcome) ist ein Kondensationsprodukt von Formaldehyd mit *p*-Methoxyphenyläthylmethylamin. Wir danken der Erzeugerfirma für die freundliche Überlassung entsprechender Versuchsmengen.
[10] G. K. STEIGLEDER, Y. KAMEI und R. KUDICKE, Arch. klin. exp. Derm. *217*, 417 (1963).

The Action of Adrenergic β-Receptor Blocking Agents on Susceptibility to Cardiac Arrhythmias in Hypothermia and Hypoxia

It has been shown in earlier experiments that enhanced susceptibility of the in situ heart to arrhythmias, as observed in the first stage of hypothermia and hypoxia, is mainly due to an increased excitatory state of the autonomic nervous centres regulating cardiac function (SZEKERES, MÉHES, and PAPP[1], SZEKERES and PAPP[2,3]). Antiarrhythmic action of the surgical and pharmacological elimination of the vagal influence in moderate hypothermia and hypoxia was clearly demonstrated in above experiments. Cardiac sympathectomy by surgical means, however, only reduced but did not entirely change back to normal the enhanced tendency to arrhythmias;

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whereas pharmacological blockade of the sympathetic innervation by Hydergin proved to be completely unsuccessful in this respect. Hydergin is known to block the adrenergic α-receptors, i.e. it does not directly influence the sympathetic regulation of the heart, whereas agents blocking adrenergic β-receptors exert their inhibiting action primarily on this latter (AHLQUIST[4,5]). Also the antiarrhythmic action of adrenergic β-receptor blocking compounds seems to be well established (FÖRSTER et al.[6], MORAN et al.[7], MORALES-AGUILERÁ and VAUGHAN WILLIAMS[8], SEKIYA and VAUGHAN WILLIAMS[9]). Therefore it seemed to us promising to study the influence of β-receptor blocking sympatholytics on the increased tendency to arrhythmias due to hypothermia and hypoxia.

Susceptibility to arrhythmias was estimated on the heart in situ of anaesthetized cats by the electrical fibrillation threshold method, i.e. the threshold strength of current required to produce auricular and ventricular fibrillation respectively. Detailed description of the method, as well as that of the cooling procedure, is given in another paper (SZEKERES, MÉHES, and PAPP[1]). Moderate arterial hypoxia was produced by inhaling a gas mixture containing 5% O_2 in N_2 for 5–8 min (SZEKERES and PAPP[2,3]). The adrenergic β-receptor blocking compounds used were: Dichloroisoproterenol (DCI), Alderline (Pronethalol, Nethalide) sec. I.C.I., Inderal (Propranolol) sec I.C.I. and two new compounds: i06 = 1(3,4-dichlorphenyl)-2-isopropylaminopropanol and i013 = 1(3,4-dichlorphenyl)-2-isopropylaminobutanol (FÖRSTER et al.[6]).

The influence of pretreatment with the adrenergic blocking agent i06 on the hypothermic decrease of the auricular and ventricular fibrillation thresholds is demonstrated in Figure 1. As seen, 3 mg/kg i06 i.v. increased fibrillation thresholds already in the normothermic animal (FÖRSTER et al.[6]) and subsequent cooling – though it somewhat decreased the elevated thresholds – was not able to reduce it down to, or below, the initial value. In contrast to the above findings after elimination of the drug, cooling of the rewarmed animal markedly diminished both thresholds below the initial value. Statistical evaluation of the experiments made with Inderal and i06 has shown (Table) that both substances significantly increased auricular and ventricular fibrillation thresholds at 28–32°C body temperature as compared to the values at 36–38°C measured prior to treatment, whereas control animals exhibited a significant decrease of the fibrillation thresholds when cooled to 28–32°C.

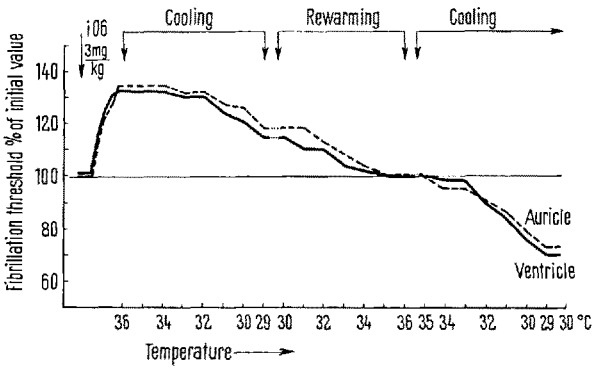

Fig. 1. Effect of a pretreatment with 3 mg/kg i06 i.v. on the auricular and ventricular fibrillation thresholds of the anaesthetized cat in hypothermia. Solid line ventricle, broken line auricle.

[4] R. P. AHLQUIST, Am. J. Physiol. *153*, 586 (1948).
[5] R. P. AHLQUIST, Arch. int. Pharmacodyn. *139*, 38 (1962).
[6] W. FÖRSTER, I. GUHLKE, J. PAPP, V. RÖSLER, and L. SZEKERES, Arch. int. Pharmacodyn., in press.
[7] N. C. MORAN, J. I. MOORE, H. K. HOLCOMB, and G. MUSHET, J. Pharmacol. exp. Ther. *136*, 37 (1962).
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Effect of Inderal (0.5 mg/kg) and 106 (3 mg/kg) administered intravenously prior to cooling on the auricular and ventricular fibrillation thresholds of the anaesthetized hypothermic cat

Pretreatment	No. of experiments	Fibrillation threshold in % of initial value[a] at			p
		36–38 °C body temperature	28–32	Difference	
Auricle					
Control	14	100.0 (0.667 mA)	80.8 ± 11.3 (0.539 ± 0.076 mA)	− 19.2 ± 3.0 (− 0.128 ± 0.020 mA)	< 0.001
Inderal	13	100.0 (0.403 mA)	153.3 ± 13.9 (0.618 ± 0.056 mA)	+ 53.3 ± 9.9 (+ 0.215 ± 0.040 mA)	< 0.001
i06	10	100.0 (0.800 mA)	128.0 ± 4.4 (1.024 ± 0.036 mA)	+ 28.0 ± 2.5 (+ 0.224 ± 0.021 mA)	< 0.001
Ventricle					
Control	14	100.0 (1.046 mA)	82.2 ± 3.2 (0.860 ± 0.033 mA)	− 17.8 ± 2.2 (− 0.186 ± 0.023 mA)	< 0.001
Inderal	13	100.0 (0.819 mA)	120 8 ± 4.2 (0.989 ± 0.034 mA)	+ 20.8 ± 3.7 (+ 0.170 ± 0.030 mA)	< 0.001
i06	10	100.0 (1.085 mA)	124.2 ± 5.3 (1.348 ± 0.058 mA)	+ 24.2 ± 2.3 (+ 0.263 ± 0.025 mA)	< 0.010

[a] Absolute values in parentheses.

The effect of pretreatment with adrenergic β-receptor blocking agent on the increased susceptibility to fibrillation in moderate hypoxia is demonstrated in Figure 2. As can be seen, hypoxia reversibly reduced auricular and ventricular fibrillation thresholds: 1 mg/kg DCI evoked a clearly demonstrable rise of both and practically prevented subsequent hypoxia in reducing the thresholds below the initial value. Adrenergic β-receptor blocking agents are effective also when given during the hypoxic period. Figure 3 shows that 2 mg/kg DCI, 2 mg/kg i06, 1 mg/kg Alderline and 2 mg/kg i013 are equally effective in counteracting the fibrillation threshold reducing influence of moderate hypoxia. This latter is reproducible at will after elimination of the drugs, as can be seen from the graph.

Accordingly β-receptor blocking sympatholytics proved to be useful in preventing hypothermic and hypoxic increase of susceptibility to arrhythmias. Whether this effect is mainly due to an inhibitory influence on the cardiac sympathetic, or to some 'unspecific' quinidine-like antiarrhythmic action of these compounds (MORALES-AGUILERÁ and VAUGHAN WILLIAMS[8], SEKIYA and VAUGHAN WILLIAMS[9]), still remains to be cleared up.

The significance of our findings can be summarized as follows: On the one hand they draw attention to a new possibility of therapeutic application of the adrenergic β-receptor blocking agents, and on the other hand they point to the important role of the cardiac sympathetic control in the mechanism of increased liability to arrhythmias in moderate arterial hypoxia and hypothermia.

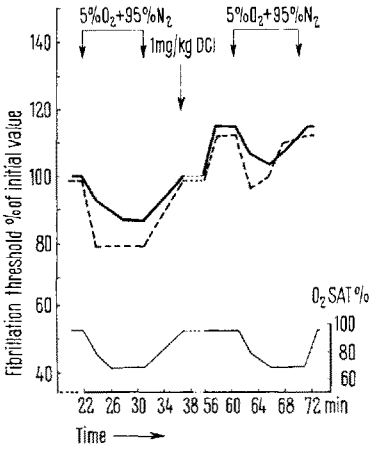

Fig. 2. Effect of DCI pretreatment (1 mg/kg i.v.) on auricular and ventricular fibrillation thresholds in arterial hypoxia.

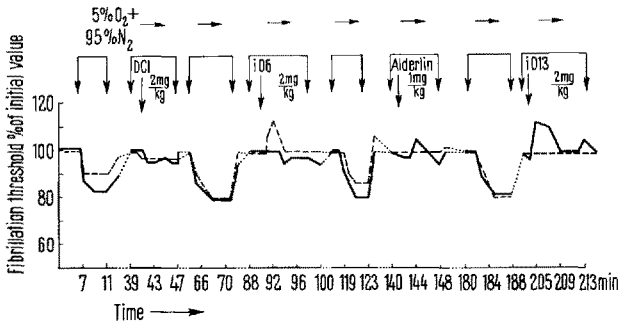

Fig. 3. Influence of different adrenergic β-receptor blocking agents on hypoxic decrease of the fibrillation thresholds, when administered i.v. during inhalation of a gas mixture containing 5% O_2 in N_2.

Zusammenfassung. Erhöhte Arrhythmiebereitschaft des hypothermischen und hypoxischen Herzens (in situ) konnte durch β-Rezeptoren blockierende Sympatholytica, wie Inderal, Alderlin und Dichloroisoproterenol, sowie zwei seiner Homologe (i06 und i013) stark bis völlig gehemmt werden.

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Department of Pharmacology of the University Medical Schools of Pécs (Hungary) and Magdeburg (DDR), June 25, 1965.

Degenerative Changes in Dendrites Following Axonal Transection

Two basic phenomena are utilized for tracing the course and termination and for localizing the cells of origin of nerve fibre bundles. These are the Wallerian degeneration and the retrograde cell reaction, respectively. Some neuroanatomical problems cannot be solved experimentally by Wallerian degeneration or by retrograde cell reaction. For example, in certain loci it is not possible to make lesions so selective that the ensuing degeneration will represent axons and axonal arborizations belonging to one fibre system only. For this reason it may be impossible to demonstrate experimentally whether degenerating arborizations passing to a nervous structure are collaterals from a neighbouring fibre system or whether they are true terminal branches belonging to a separate system.

Utilizing the fact that nerve cells of new-born animals are more apt to react by disintegration following axonal transection than nerve cells of adult animals[1], the author has performed a series of experiments.

In one group of kittens, unilateral transections of the hypoglossal nerve were carried out. The animals were operated on at various ages and kept alive for various

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