adrenergic nerves. 6-OH-DA itself does not give rise to any formaldehyde-induced fluorescence[13].

The autoradiographs (Figures 2 and 4) showed, after incubation of intact irides in $10^{-5}M$ ^{3}H-6-OH-DA, an accumulation of grains in a pattern very closely resembling the morphology and distribution of the adrenergic nerves as demonstrated histochemically (Figures 3 and 5). Denervated irides did not disclose this accumulation but just a diffuse distribution of grains in a number similar to that observed between the accumulations of grains in the intact irides. It was not possible to demonstrate any accumulations of grains after incubation in $10^{-6}M$ ^{3}H-6-OH-DA probably due to the low specific activity of the ^{3}H-6-OH-DA used. Occasionally, accumulations of grains were observed in structures possibly representing mast cells.

Discussion. The results presented show that 6-OH-DA can be taken up and accumulated in adrenergic nerves. The evidence comes from the observations that after incubation in ^{3}H-6-OH-DA the accumulation of grains have a distribution practically identical with that of the adrenergic nerves as revealed by fluorescence histochemistry. Furthermore, this accumulation can be prevented by sympathetic denervation. The grains observed spread diffusely in between the network represents the extraneuronal uptake of 6-OH-DA. At least part of the extraneuronal uptake might, however, also be localized in mast cells. 6-OH-DA in all probability uses the very efficient axonal 'membrane pump' for its inward transport and accumulation in the adrenergic neuron, since blockade of this uptake-accumulation mechanism either by desipramine or by incubation the tissue at 0 °C strongly reduces 6-OH-DA uptake[13] and prevents degeneration[7,13,18]. This property of 6-OH-DA certainly explains the fact that it produces a selective destruction of the adrenergic nerves[6,8]. Although it has been shown that 6-OH-DA can be taken up in the amine-storage granules intraneuronally, this does not seem to be a prerequisite for inducing degeneration[13,18,19].

Zusammenfassung. Die Regenbogenhaut der Maus wurde in einer physiologischen Pufferlösung mit radioaktivem 6-Hydroxydopamin (^{3}H-6-OH-DA) inkubiert. Mit Hilfe autoradiographischer und fluoreszenz-histochemischer Untersuchungen konnte gezeigt werden, dass ^{3}H-6-OH-DA in die adrenergischen Nervenfasern der Regenbogenhaut aufgenommen und gespeichert wird. Dieser Befund bringt die direkte Erklärung der durch 6-Hydroxydopamin induzierten Degeneration der adrenergen Fasern.

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Influence of Coronary Dilators on the Uptake of Serotonin by Human Platelets and Adrenaline by Chromaffine Granules

Many drugs with different chemical structures have been used in the treatment of angina pectoris. Whereas their therapeutic value appears to be limited[1], there is no doubt about the ability of some of these compounds to interfere with adrenergic mechanisms[2], especially with the transport of catecholamines through subcellular and cellular membranes[3-6]. Since the membrane of human blood platelets shares certain properties with the axoplasma membrane of the adrenergic neuron, e.g., the active transport of biogenic amines[7], this model was chosen to investigate the effect of the 4 coronary dilators (see formula) prenylamine (Segontin®), oxyfedrine (Ildamen®), verapamil (Isoptin®) and carbochromenum (Intensain®) on the uptake of serotonin by human platelets. For comparison, the influence of these compounds on the uptake of adrenaline by chromaffine granules was examined.

Materials and methods. 1. Uptake of ^{3}H-serotonin by human platelets. Blood was obtained from healthy donors by venous puncture with a plastic-syringe containing 1 ml 3.8% sodium citrate per 10 ml blood. Platelet-rich plasma was prepared as described by Zieve et al.[8]. Samples of 0.2 ml platelet-rich plasma containing about $4–6 \times 10^7$ platelets were incubated at 37 °C with 0.1 ml (1 μC) of ^{3}H-serotonin (8.6 C/mM; Radiochem. Ceutre, Amersham) and 0.7 ml Krebs-Ringer-bicarbonate buffer (without Ca^{++} and Mg^{++}) pH 7.4. The drugs were dissolved in buffer. Control samples were prepared by addition of ^{3}H-serotonin to the platelets at 4 °C immediately before centrifugation. After incubation, the platelets were sedimented at $1200 \times g$ for 20 min and supernatants decanted. The sediments were lysed in 1 ml 0.01 n HCl at 37 °C with shaking. Radioactivity was determined as previously described[3].

2. Uptake of ^{14}C-adrenaline by chromaffine granules. Granules from bovine adrenal medulla were prepared

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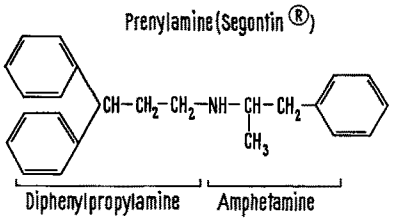
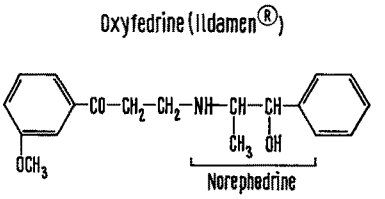
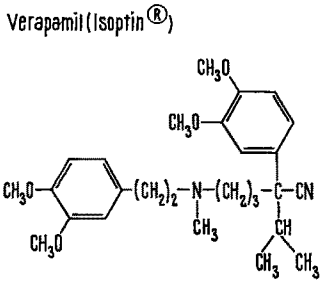
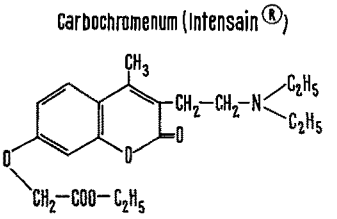
by high speed centrifugation of homogenates[9]. Incubation of the isolated granules was carried out as described by CARLSSON et al.[10] at 37 °C for 15 min in glycylglycine buffer pH 7.3 containing 0.0025 M ATP and $MgCl_2$ and 0.1 μC ^{14}C-adrenaline (20 mC/mM; New Engl. Nucl. Corp.) without shaking.

Results. 1. Effect on uptake of ^{3}H-serotonin by platelets. Human platelets concentrated the labelled serotonin against a gradient[11,12]. The time curve of uptake obtained under our experimental conditions is shown in Figure 1. A steady state distribution was reached after 40 min. Prenylamine and oxyfedrine inhibited the uptake significantly in concentrations of 10^{-6} and 10^{-5} M resp. especially during the initial 5–10 min when a nearly linear uptake occurred. Similar results were obtained with verapamil (5×10^{-5} M) and carbochromenum (10^{-4} M).









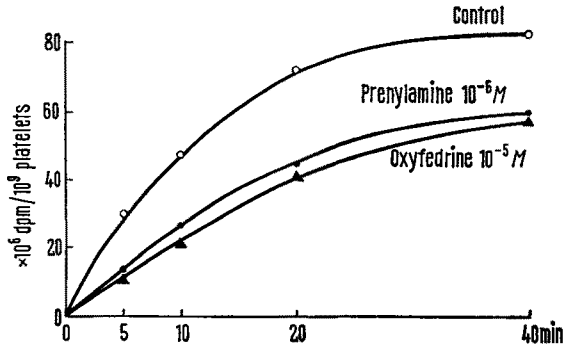


Fig. 1. Time curve of serotonin uptake by human platelets. Inhibition of serotonin uptake by 10^{-6} M prenylamine (●—●) and 10^{-5} M oxyfedrine (▲—▲). For details see text.

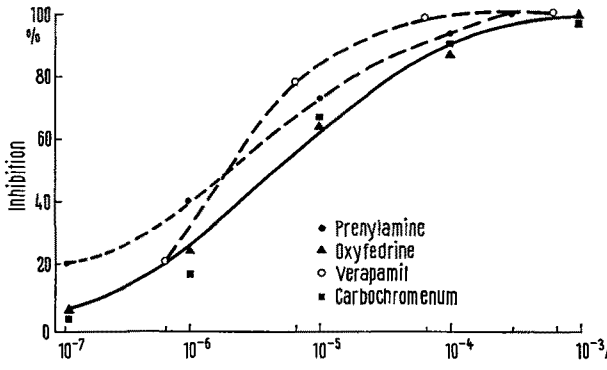


Fig. 2. Concentration-response curves of inhibition of serotonin uptake by human platelets in the presence of various coronary dilators. Prenylamine (●—●), oxyfedrine (▲—▲), verapamil (○—○), carbochromenum (■—■).

Therefore, dose effect curves were established after incubation for 5 min at 37 °C in the presence of increasing concentrations of the drugs. As depicted in Figure 2, prenylamine and verapamil were the most active inhibitors of serotonin uptake, whereas oxyfedrine and carbochromenum were about 2 times less potent, if one compares the concentrations causing a 50% inhibition of uptake.

2. Effect on uptake of ^{14}C-adrenaline by granules. The accumulation of ^{14}C-adrenaline by the granules was rapid during the first 15 min of incubation and the inhibitory effect of the drugs on ^{14}C-adrenaline uptake could be well observed under these conditions. For a 50% inhibition, concentrations of 0.2 up to 10×10^{-5} M of the compounds tested were necessary (Table). Prenylamine was the most potent drug followed by oxyfedrine, verapamil, and carbochromenum.

Discussion. Prenylamine is chemically a derivative of amphetamine, oxyfedrine a derivative of norephedrine. Both amphetamine and norephedrine are able to inhibit the uptake of noradrenaline at cellular and subcellular membranes[13]. Verapamil and carbochromenum show little (–CH_2–CH_2–NH–) or no similarity with compounds known to possess sympathomimetic activity. From the lack of free phenolic hydroxy groups, high lipid solubility and surface activity, i.e., a high affinity of these drugs to various kinds of membranes should be assumed. Such compounds are known to lower surface tension efficiently

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and dose-dependently by accumulation at the water/air interface[5,14]. In high concentrations, prenylamine destroys the membranes of erythrocytes, chromaffine granules, mast cells, and probably also of platelets – which results in the release of hemoglobin, catecholamines, histamine, and serotonin, respectively[14–17]. In low concentrations, the drugs produce a membrane 'stabilising' effect[18]. Electron micrographs showed swelling of the membranes of mast cells and chromaffine granules. This effect was temperature-independent and therefore should be brought about by a non-energy-requiring mechanism of action[14,16].

Consequently, inhibition of uptake of serotonin and adrenaline or swelling of serotonin storing granules of blood platelets and chromaffine granules can be assumed to be the result of a nonspecific, merely physicochemical interaction of the drugs investigated with subcellular and cellular membranes.

Concentration causing 50% inhibition of ^{14}C-adrenaline uptake by isolated chromaffine granules (cattle)

	$\times 10^{-5} M$
Prenylamine (Segontin®)	0.2
Oxyfedrine (Ildamen®)	1
Verapamil (Isoptin®)	4
Carbochromenum (Intensain®)	10

Glycylglycine pH 7.3, ATP and $MgCl_2$ 0.0025 M, 37 °C 15 min.

Zusammenfassung. In einer unspezifischen, physikalisch-chemischen Wirkung von vier oberflächenaktiven Coronardilatatoren auf die Membranen von menschlichen Thrombozyten und isolierten chromaffinen Granula wird die Ursache für die Hemmung der Aufnahme von Serotonin bzw. Adrenalin erblickt.

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Mesothelzellveränderungen nach der intraperitonealen Injektion von denaturiertem Phytohaemagglutinin[1]

Natives Phytohaemagglutinin (PHA) induziert in peritonealen Zellen eine Produktion von Hyaluronsäure, die mit morphologischen Mesothelzellveränderungen korreliert ist (Mohr et al.[2a,b]). Dieser Reaktion folgt eine Proliferationswelle im Mesothelzellverband (Mohr et al.[2c]). Hitzedenaturiertes PHA führt weder zu einer Transformation der Lymphozyten in vitro (Nowell[3]), noch hat es einen Einfluss auf die primäre Immunantwort gemessen an der Anzahl der plaquebildenden Zellen (Spreafico und Lerner[4]). In den lymphatischen Organen führt denaturiertes PHA im Gegensatz zum nativen PHA nicht zur Vermehrung undifferenzierter Zellen (Machado et al.[5]). Daher sollte in den vorliegenden Untersuchungen die Reaktion der Peritonealzellen auf die i.p. Injektion von hitzedenaturiertem PHA ermittelt werden.

Material und Methode. Phytohaemagglutinin (PHA-P, Difco Laboratories, Detroit) wurde nach der Rehydrierung mit Aqua dest. (5 ml Aqua dest. pro Ampulle Trockensubstanz PHA-P) 30 min bei 120 °C autoklaviert. Von dieser Lösung erhielten 5 männliche, 4 Wochen alte SIV-50 Ratten (S. Ivanovas, Med. Versuchstierzuchten, Kisslegg/Allgäu) mit einem Durchschnittsgewicht von 70 g je 1,0 ml i.p. injiziert. 3 Kontrolltiere blieben unbehandelt. 48 h nach der Injektion wurden die Tiere in Äthernarkose dekapitiert, da nach vorausgegangenen Untersuchungen mit nativem PHA die morphologischen Veränderungen zu diesem Zeitpunkt am stärksten sind und sich ein hoher Gehalt an Hyaluronsäure in der Peritonealflüssigkeit nachweisen lässt (Mohr al[2a,b]).

In der Peritonealflüssigkeit wurde der Hyaluronsäuregehalt chemisch bestimmt[6]. Weiterhin wurden Ausstrichpräparate der Peritonealflüssigkeit hergestellt und nach Pappenheim gefärbt. Vom parietalen und diaphragmalen Peritonealmesothel wurden nach der Methode von Beneke et al.[7] Häutchenpräparate angefertigt, die mit Haematoxylin-Eosin gefärbt wurden. In den Ausstrichpräparaten wurden die Zellen differenziert, die Häutchenpräparate wurden auf morphologische Veränderungen untersucht.

Ergebnisse. 1. Peritonealflüssigkeit. Während die Peritonealflüssigkeit von Kontrolltieren keine neutrophilen Granulozyten enthält, fand sich 48 h nach der Injektion von denaturiertem PHA eine neutrophile Reaktion (Tabelle). Die Rundzellen zeigten meist einen breiten basophilen Zytoplasmasaum. Vereinzelte Rundzellen enthielten solitäre Zytoplasmavakuolen oder phagozytierte Kernreste neutrophiler Granulozyten. Hyaluronsäure konnte in der Peritonealflüssigkeit chemisch nicht nachgewiesen werden.

2. Mesothelzellen. Unter physiologischen Bedingungen wird das parietale Peritoneum von einem einschichtigen geschlossenen Mesothelzellverband mit meist nicht sicht-

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