In vitro Lysis of Erythrocytes and Chromaffine Granules by Prenylamine

Prenylamine (Segontin®) releases catecholamines from chromaffine medullary granules as well as from sympathetic nerve vesicles[1-4]. The i.v. injection of the drug (5 mg/kg) in rats produced strong hemoglobinuria[5,6]. Thus, prenylamine is obviously able also to release hemoglobin from erythrocytes.

The present paper gives evidence for a common mechanism underlying both the release of amines from chromaffine granules and of hemoglobin from erythrocytes.

Material and methods. Granules from bovine adrenal medulla were prepared by high speed centrifugation of homogenates as previously described[7]. Incubation of the isolated granules (1 ml granule suspension containing 2.5–3 mg protein in samples of 5 ml) was carried out at 37 °C by shaking in an isotonic medium containing 0.3 M sucrose and $10^{-3}M$ Mg^{++}. *Tris*-buffer was added to obtain pH 7.2. DL-prenylamine lactate (Farbwerke Hoechst) was dissolved in the buffered sucrose.

Determination of total catecholamines[8] in the granules was performed after sedimentation (15,000 g, 20 min) and extraction with 0.4 N $HClO_4$.

Electron micrographs from granules were obtained after fixation with glutaraldehyde and post-fixation with 1% OsO_4. For details see [9].

Human and rat erythrocytes washed 3 times with saline-sodium acetate buffer pH 7.5 (0.81% NaCl, 0.198% sodium acetate) were suspended in the same buffer to give a final concentration of 5%. Prenylamine was added in increasing amounts. After 30 min incubation at 37 °C with shaking, the suspensions were centrifuged (1150 g). The released hemoglobin was estimated in the supernatant[10].

In other experiments the erythrocytes were suspended in hypotonic saline-sodium phosphate buffer (pH 7) according to SEEMAN and WEINSTEIN[11] in order to obtain a 50% hemolysis after 5 min standing at room temperature. Prenylamine in increasing amounts was previously added.

Lowering by prenylamine of surface tension (aqua bidest.) was measured at 37 °C by means of an interfacial tensiometer according to LE COMTE DU NOÜY (Fa. Krüss, Modell K 8600).

Results. (1) As can be seen from Figure 1A, surface tension was lowered markedly by prenylamine at concentrations between 10^{-4} and $10^{-3}M$. In the same range of concentrations amphetamine which provides one section of the prenylamine molecule was without any effect.

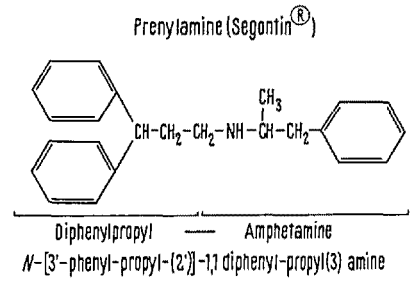
Figure 1B shows that complete hemolysis of rat and human erythrocytes occurred on incubation with prenylamine at final concentrations of 2–3 $\times$ $10^{-4}M$. Somewhat lower concentrations (6×10^{-5} to $10^{-4}M$) caused total depletion of catecholamines from isolated chromaffine granules. The release was nearly the same at 22° and 37 °C (Figure 1B).

(2) Electron micrographs of granules incubated with prenylamine at these concentrations demonstrate that the granules were swollen and their membranes severely damaged (Figures 2A and B).

Amphetamine and methamphetamine, even at concentrations of 2–4 $\times$ $10^{-3}M$ produced a release of catecholamines of not more than 50%. This effect was obtained only when the sucrose medium contained sodium phosphate (0.3 M sucrose + 66 mM sodium phosphate buffer, pH 6.8; 4:1 v/v). Under these conditions no damage of the granule membranes could be detected by electron microscopy.

(3) On the other hand, prenylamine protected erythrocytes against hemolysis induced by hypotonic saline. Stabilization was maximal at concentrations of 2 to $3 \times 10^{-4}M$ (Figure 3), i.e. at concentrations which caused complete hemolysis of erythrocytes suspended in isotonic saline (cf. Figure 1B).

Discussion. Prenylamine may be defined chemically as a N-diphenylpropyl-substituted derivative of amphetamine (see Formula). Therefore the drug should behave pharmacologically as an indirect-acting sympathomimetic amine because of the amphetamine moiety of the molecule and exert adrenergic actions[5,6]; on the other hand, the



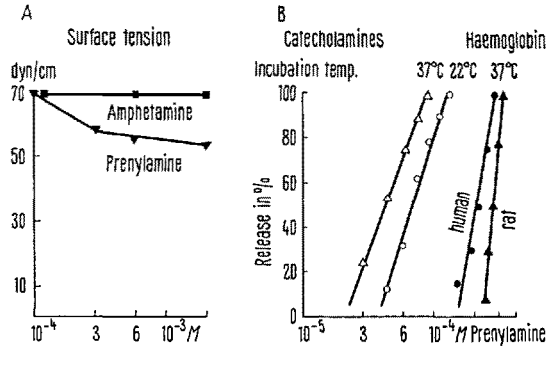


Fig. 1. (A) Lowering by prenylamine of surface tension. (Aqua bidest. at 37 °C = 70.4 dyn/cm). (B) Release by prenylamine of catecholamines from isolated chromaffine granules (bovine adrenal medulla) and of hemoglobin from human (●—●) and rat (▲—▲) erythrocytes. Concentration-response curves.

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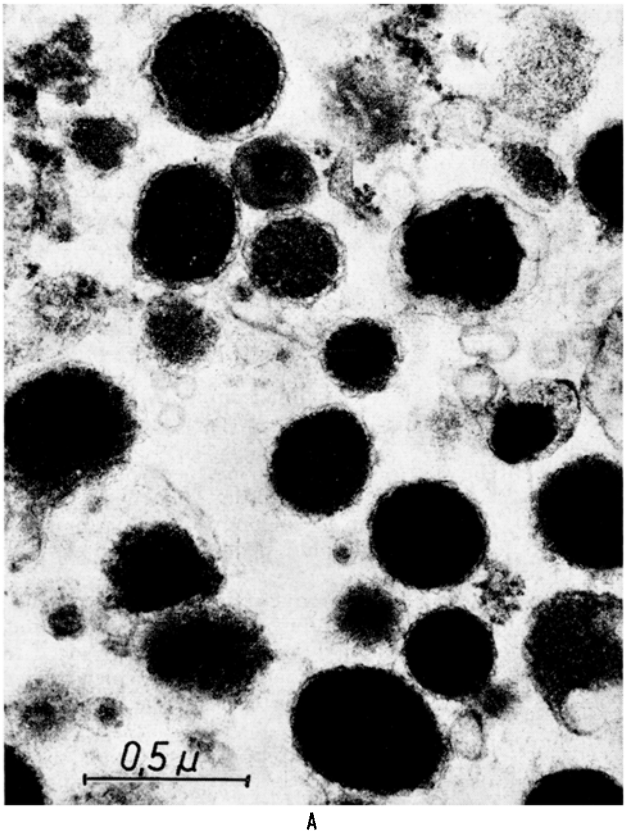
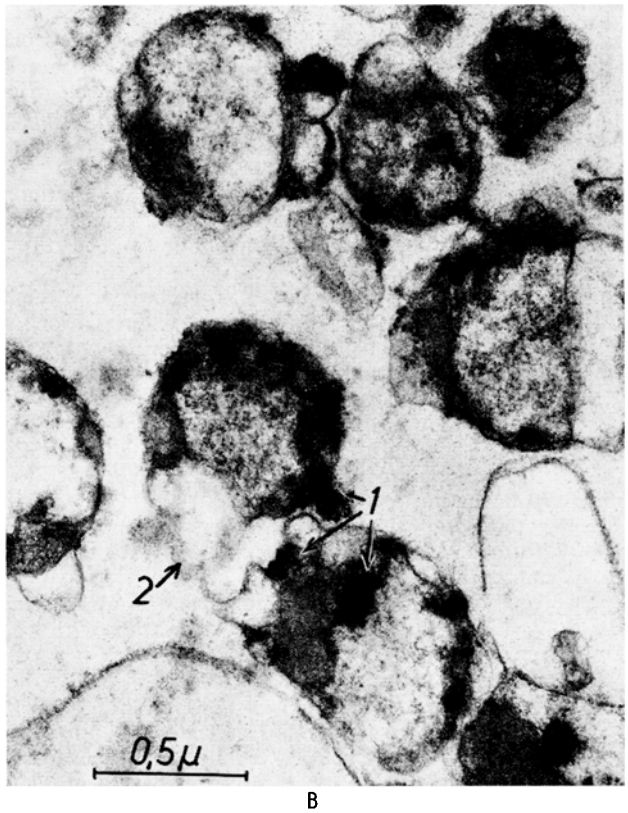


A



B

Fig. 2. (A) Electron micrograph of chromaffine granules suspended in 0.3 M sucrose (controls). (B) After incubation with prenylamine ($2.4 \times 10^{-4} M$, 30 min, 37 °C). (A) and (B) with the same magnification ($\times$ 50,000). In (B) granules are swollen and contain only small islets of electron dense material (1). Their membranes are severely damaged (2).

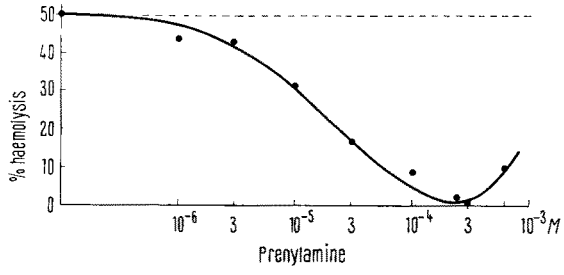


Fig. 3. Protection by prenylamine of human erythrocytes against hypotonic hemolysis (50%). (For details see 'Methods').

diphenylpropyl moiety may enable prenylamine to exert rather unspecific, e.g. spasmolytic[5,6], local anaesthetic[12] and quinidine-like[13] effects. In the urine of man and rat amphetamine could be demonstrated as a metabolite of prenylamine[14].

The lysis of erythrocytes and chromaffine granules caused by prenylamine as shown in the present paper was obviously due to the diphenylpropyl moiety of the molecule which provides the drug with high lipophilic and surface active properties. The slope of the dose-response curves for both the hemolytic and granulolytic action was very steep, corresponding to a nearly 'all or nothing effect', due to disruption of the membranes. This may explain why prenylamine released from chromaffine granules not only catecholamines but also ATP – an action shared by reserpine[2,3] which, interestingly, also proved to be a hemolytic agent[11] like prenylamine in our experiments.

The action of prenylamine on membranes was similar to that exerted by detergents like saponin. Saponin released catecholamines from medullary granules[5] and hemoglobin from erythrocytes[15]. However, in contrast to saponin, which is a 'specific' hemolytic agent[14], prenylamine – like reserpine, chlorpromazine and other 'un. specific' hemolytics[16] – protected erythrocytes against hemolysis induced by hypotonic saline as shown in the present paper. In this context, it is of interest that prenylamine as well as reserpine in low concentrations (10^{-5} to $10^{-6} M$) were able to inhibit the spontaneous release of catecholamines from isolated granules of sympathetic nerves[2] and ganglia[17]. In the same range of concentrations these drugs inhibited the 'ATP-Mg^{++}-dependent uptake' of catecholamines into chromaffine granules[18,19].

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An unspecific (temperature-independent) mechanism seems to underly these 'membrane effects' exerted by prenylamine and other surface active drugs.

In low concentrations they will exert a protective action on the particles, i.e. an inhibition of the spontaneous release of catecholamines from isolated granules and of hemoglobin from erythrocytes. The reason could be an enlargement of the surface area/volume ratio as shown by SEEMAN[15] for erythrocytes which were incubated with prochlorperazine, and as demonstrated in the present paper by Figure 2B for chromaffine granules which were swollen after incubation with prenylamine. This enlargement is possibly also responsible for the inhibition of the ATP-Mg^{++}-dependent uptake mechanism localized in the membranes of the granules.

With high concentrations of the corresponding drugs the surface area/volume ratio of the particles is obviously enlarged to such an extent that disruption of the membranes and complete release of the contents will result.

Zusammenfassung. In der Oberflächenaktivität des Prenylamins, bedingt durch den lipophilen Diphenylpropylanteil des Moleküls, wird die Ursache dafür erblickt, dass hohe Konzentrationen zur Freisetzung von Brenzcatechinaminen aus chromaffinen Granula sowie von Hämoglobin aus Erythrocyten führen. Elektronenmikroskopische Aufnahmen zeigten schwere Membranschädigungen der mit Prenylamin inkubierten Granula. Prenylamin hemmte bzw. verhinderte die Hämolyse der in hypotonischer Salzlösung suspendierten Erythrozyten. Ein gemeinsamer Mechanismus, der sowohl die granulolytische und hämolytische als auch die protektive Wirkung auf Granula- und Erythrozytenmembran erklären könnte, wird diskutiert.

H. GROBECKER, P. HOLTZ,
D. PALM, I. J. BAK
and R. HASSLER

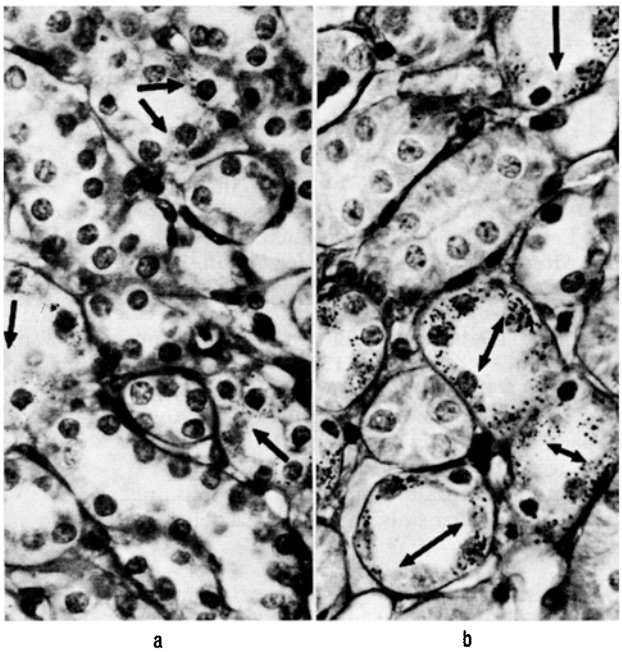
Pharmakologisches Institut der Universität und Max-Planck-Institut für Hirnforschung, Neuroanatomische Abteilung, 6 Frankfurt a.M. (Germany),
28 March 1968.

Änderungen des Glykogengehaltes in der Niere von Ratten nach Injektionen von Na_2[Ca-ÄDTA]

Im Laufe einer eingehenden histologisch-histochemischen Studie der Genese der Nierenschäden, wie sie durch therapeutische Chelatbildner hervorgerufen werden, ergab sich ein Befund, der in der bisherigen Literatur[1–9] noch nicht beschrieben wurde und wegen der Aktualität der Problematik kurz mitgeteilt werden soll.

16–20 Wochen alte Rattenmännchen des Heiligenberg-Stammes von 230–300 g Gewicht erhielten täglich i.p. 8, 4 oder 2 mmol Na_2[Ca-ÄDTA]/kg injiziert, die Kontrolltiere äquimolare NaCl-Lösung. Jeweils 24 h nach der letzten Injektion wurden je 5 Versuchs- und Kontrolltiere getötet, aus einer Niere eine Querscheibe herausgetrennt, in Rossmanscher Lösung fixiert[10], mittels Tetrahydrofuran entwässert, in Paraplast eingebettet und 7 μ dick geschnitten. An histologisch gefärbten Schnitten lassen sich die gleichen Gewebsveränderungen feststellen, die auch von anderen Untersuchern beschrieben und als hydropische Degeneration bezeichnet wurden. Ausserdem wurde die PJS-Färbung nach MCMANUS angewandt[10]. Die Spezifität der Glykogendarstellung ergab sich durch die Diastasebehandlung von Parallelschnitten, die 1 h bei 37 °C einer 1prozentigen phosphatgepufferten (pH 6,8) Diastase-(Merck)-Lösung ausgesetzt waren, während sich die Vergleichsserie entsprechend in Phosphatpuffer befand[10].

Zahl und Höhe der NaCl-Injektionen verursachen keine augenfällige Beeinflussung des Wohlbefindens der Kontrolltiere. In den Sammelrohrepithelien der Niere lassen sich immer (wie auch bei unbehandelten Tieren) vereinzelte kleine Glykogengranula nachweisen (Figur 1a).



a b

Fig. 1. (a) Kontrolle: 8 × 4 mmol NaCl/kg/d. PJS-Färbung-Weigert: → Glykogen in Sammelrohren. × 500. (b) Versuch: 4 × 4 mmol Chelat/kg/d. Sonst wie (a).

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