Influence of pronethalol on the effect of various hypotensive agents in the renal hypertensive rat

Treatment mg/kg·day		n	Control value mm Hg	Mean blood pressure values (mm Hg) on 3rd and 4th day of treatment	
				Acute effect measured 2 h after last injection	Prolonged effect measured 24 h after last injection
Saline control		4	195 ± 5	186 ± 4	187 ± 2
Pronethalol	10 s.c.	8	193 ± 2	186 ± 4	173 ± 4
Hydralazine	6 p.o.	7	198 ± 7	133 ± 8	152 ± 9
Hydralazine + Pronethalol	6 p.o. 10 s.c.	8	195 ± 6	186 ± 5	160 ± 7
Hydralazine	15 p.o.	7	192 ± 6	113 ± 10	151 ± 11
Hydralazine + Pronethalol	15 p.o. 10 s.c.	7	194 ± 6	154 ± 11	148 ± 10
Guanethidine	6 s.c.	7	198 ± 6	122 ± 5	133 ± 7
Guanethidine + Pronethalol	6 s.c. 10 s.c.	6	195 ± 7	167 ± 5	132 ± 4
α-Methyl-DOPA	300 p.o	13	188 ± 5	143 ± 3	152 ± 3
α-Methyl-DOPA + Pronethalol	300 p.o. 10 s.c.	7	191 ± 7	146 ± 7	148 ± 7

Treatment daily for 4 days. Blood pressure measurement twice daily, immediately before and 2 h after treatment, by tail plethysmography. Values given are means ± SE.

action was found. This may be due to the relatively short duration of action of pronethalol. It is possible that pronethalol, given in higher doses and at shorter intervals, could inhibit the prolonged as well as the acute hypotensive effects.

Hydralazine and guanethidine produce – as far as is known – a reduction in peripheral resistance and a fall in blood pressure by different mechanisms[4–6]. The inhibition of their hypotensive effects by pronethalol indicates, however, that they probably have some common peripheral or central mechanism of action. It is, as yet, not possible to decide between these two possibilities.

Although preliminary experiments with other β-receptor blocking agents show the same results, the inhibition of the effects of hypotensive drugs by pronethalol may not be related to its β-receptor blocking activity.

Zusammenfassung. Die hypotensiven Wirkungen von Hydralazin und Guanethidin an renal hypertonischen Ratten konnten für kurze Zeit durch Pronethalol antagonisiert werden. Die Wirkung von α-Methyl-DOPA wurde nicht beeinflusst.

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PRO EXPERIMENTIS

A Rapid Technique for Characterizing Metal-Protein Complexes by pH-Metry

The pH-metric method has been successfully used to study the hydrogen ion binding capacities of various proteins. The technique has, however, not been employed so far to investigate metal-protein interaction. In this communication the results of such studies on cobalt and nickel complexes of transfusion gelatin are reported.

MALIK et al.[1] have found that the equation[2]:

$$\mathrm{pH} - \log \frac{v_i}{n_i - v_i} = (\mathrm{pK}_{int})_i - 0.868\, Z\, W$$

which represents a titration curve in absence of metal ion for globular protein may also be applied to collagen type of protein. Since, in the presence of metal ions, a fraction of the acidic groups will be removed from participation in hydrogen ion equilibria by metal binding, it is possible to calculate the binding data directly from the difference in hydrogen ion titration curves in the presence and absence of metal ion, just as in the case of BJERRUM's[3] method.

Solutions of cobalt and nickel were prepared by dissolving chemically pure sample (A.R.) of the chloride salts in

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triply distilled water, and their contents were estimated by the usual methods. pH measurements of the mixtures of cobalt-transfusion gelatin, nickel-transfusion gelatin and transfusion gelatin alone with varying amounts of hydrochlorid acid, were carried out at 25°C on Beckman Model G pH-meter. Total ionic strength of the mixtures was adjusted to 0.15 by the addition of the requisite amounts of potassium chloride solution. Transfusion gelatin[4] supplied by the Director, National Chemical Laboratory, Poona (India), was used throughout these investigations. The Figure represents the titration curves of all three mixtures. The TANFORD method[2] was employed for the construction of such plots.

The hydrogen ion titration curves of transfusion gelatin in the presence and absence of metal ions are strikingly different. Assuming that the metal ions compete with the hydrogen ions for common site, the following conclusions may be arrived at.

(1) The amount of hydrogen ions given out by the protein is greater in the presence of metal ions than with the protein alone. This would be the case if replacement of hydrogen ions by metal is visualized from the site under consideration.

(2) The amount with which a particular metal ion displaces the hydrogen ion equilibria of gelatin towards the basic side of the functional groups may be taken as a measure of the extent of metal-protein combination. Further, since one-to-one binding is favoured in preference to intramolecular cross linking[5], the number of hydrogen ions displaced per protein molecule (as read off from the titration curve) yields directly the binding data, '$\bar{\nu}$', the number of active sites covered by metal ions. Such data are given in the Table.

Since the changes which occur during the titration of metal-protein mixtures are mostly in the pH range 3 to 5.5, where carboxyl groups are expected to lose their proton[1], it may be concluded that the carboxyl groups of aspartyl and glutamyl residues offer the principal site for the binding of both the metal ions. The values of log K computed from the SEATCHARD equation[6] come out at 2.02 and 1.86 for cobalt and nickel complexes respectively, which are almost the same as for the association of one molecule of acetic acid with cobalt[7] and nickel[8,9].

The free energy change of the combination at 25°C comes out at -2.755 and -2.537 Kcal for cobalt and nickel complexes respectively[10].

pH	H^+ dissociated in presence of metal ions	H^+ dissociated in absence of metal ions	Difference of $\bar{\nu}$	Free metal ions at equilibrium $\times 10^{-3} M$	log K
3.0	5.0	4.0	1	–	–
4.0	34.0	30.0	4	–	–
5.0	65.0	60.0	5	–	–
5.5	74.0	69.0	5	0.6	2.02
6.0	78.0	74.0	4	–	–
Concentration of nickel chloride			$1.008 \times 10^{-3} M$		
3.0	4.0	4.0	0	–	–
4.0	32.0	30.0	2	–	–
5.0	64.0	60.0	4	–	–
5.5	73.0	69.0	4	0.688	1.86
6.0	78.0	74.0	4	–	–

Concentration of cobalt chloride = $1.0 \times 10^{-3} M$.
Concentration of transfusion gelatin = $0.8 \times 10^{-4} M$.

Zusammenfassung. Die pH-Messmethode zur Bestimmung der Bindung von Wasserstoffionen an Eiweiss wurde für den Bereich der Metallionen erweitert. Die Daten über die Metallionenbindung von Kobalt und Nickel für die Transfusionsgelatine wurden aus der Verschiebung der pH-Titrationskurven bei Anwesenheit bzw. Abwesenheit der Metallionen gewonnen.

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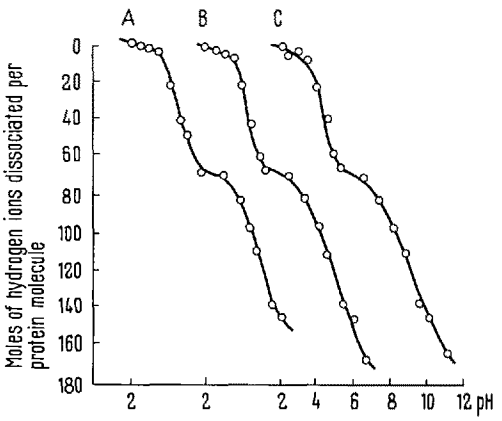

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