hand, a large variety of other actions of strychnine have been demonstrated with higher doses, namely an antagonism to the inhibitory effects of GABA[15,16], and a curare-like action in sympathetic ganglia and in neuromuscular synapses[17,18], D-tubocurarine being itself an excitatory agent when applied to the cerebral cortex[19], possibly also acting there as anti-GABA compond[20].

Strychnine at concentrations higher than 10^{-3} M apparently also expands plasma membranes[21]. This latter effect, because of the very large concentrations needed, does not appear to be related to the anticholinesterase nor to the in vivo effects of strychnine. On the other hand, a discrimination between the anticholinesterase, the anti-GABA and the curare-like effects cannot be made on the basis of concentration, since all occur at similar ones. However, the relative inefficacy as neuronal excitors of anticholinesterases more potent than strychnine[22,23], make it unlikely that the anti-AChE action of this alkaloid is the main mechanism for its excitatory effects when applied topically to the brain cortex, although it could contribute in some degree to this highly complex effect[24], since it is known that acetylcholine, which is a minor cortical transmitter, has predominantly excitatory effects on this structure[25,26]. Since nicotinic inhibitory receptors in the cerebral cortex are yet to be found[27], and D-tubocurarine itself has some anti-GABA effects[20], it seems probable that the primordial mechanism for the excitatory action of strychnine in the cerebral cortex is its anti-GABA activity.

Resumen. La estricnina inhibe competitivamente a la acetilcolinesterasa (AChE, acetilcolina acetil-hidrolasa, E.C. 3.1.1.7) y a la colinesterasa (ChE, acilcolina acil-hidrolasa, E.C. 3.1.1.8) de cerebro de rata. Se calculó un K_i de 7×10^{-5} *M* para AChE y de 1.7×10^{-4} *M* para ChE cuando sus actividades enzimáticas se midieron con acetilbetametilcolina y butirilcolina, respectivamente. Se analizó la relevancia de estos hallazgos para el mecanismo de acción de dosis altas de estricnina in vivo, concluyéndose que esta acción anticolinesterásica es de escasa importancia.

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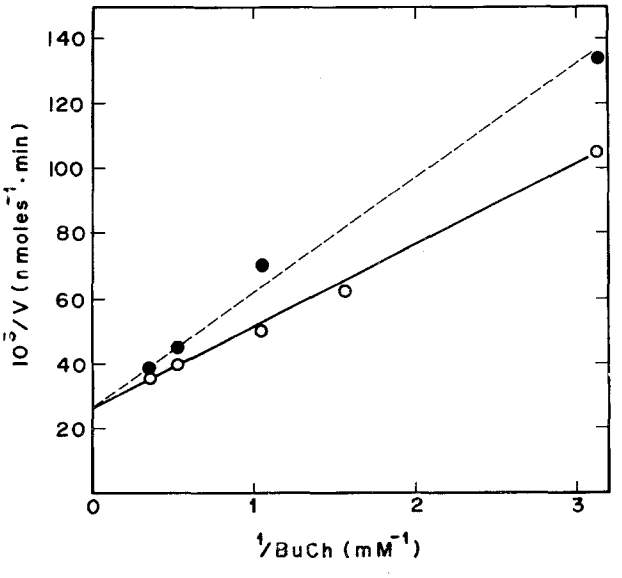

Fig. 3. Lineweaver-Burk Plot of ChE. ○, enzyme alone; ●, enzyme plus 5×10^{-5} *M* strychnine.

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[28] Acknowledgements. We are grateful to O. PETIT for technical assistance, to Dr. B. Cassels for the chromatographic analysis of strychnine, to Dr. J. E. ALLENDE for the loan of the Radiometer Titrator and to Cristina Maureira for her secretarial help. Supported by Grants from NIMH No. I RO3 MH 19125 CONICYT (project No. 80) and by Comisión de Investigación Científica, Universidad de Chile (Project No. 95).

Inhibition of Vasopressin-Induced Morbid Effects on the Rat Kidney by Pindolol and Propranolol

Studies on renal blood flow have shown that low amounts of vasopressin diminish the circulation in the renal medulla of the rat[1] and hamster[2]. From this finding a selective sensitivity to ADH of arteries of this area has been suggested[1].

In recent experiments in our laboratories, it has been observed that vasopressin, in doses nearly proportional to those which have been occasionally used in man[3], induces rat renal ischemic lesions localized in the region between medulla and cortex[4], supporting also the selective arterial sensitivity.

Since some reports indicate that in the ADH effect in animals[5,6] and in man[7] adrenergic mechanism is involved the present experiments were designed to examine whether the vasopressin-induced rat kidney damage is influenced by β-adrenoceptive receptor blocking drugs.

Material and method. The experiments to be described were conducted on young adult albino rats (Wistar origin) of both sexes, averaging 220–240 g, housed 4 in each cage, at 21–22 °C and air humidity 48–51%, 10–12½ h light/day, on pellets diet (Zootrofiki Athens-Greece) and tap water ad libitum; food was only removed 12 h before the experiment.

The antidiuretic hormone (Vasopressin-Sandoz) was injected i.p. once daily at a dose of 100 mU/100 g body wt. for 3 successive days. As β-adrenergic receptor blocking

agents, we used pindolol and propranolol, injected identically at the dose of 20 μg and 30 μg per 100 g body wt. respectively. When animals were treated with both substances, β-receptor antagonists were injected first into the right iliac region and vasopressin 15 min later into the left.

All animals were sacrificed 24 h after the last injection by a sharp blow on the head. Both kidneys were taken for histological examination and kidneys tissue blocks were fixed with neutral buffered formaldehyde in saline; paraffin-wax-embedded sections 5 μm thick were stained with heamatoxylin and eosin and 4 slides from each kidney were chosen by a blind procedure and examined randomly by light microscopy. The degree of kidney damage was assessed from inspection of 8 random fields per slide.

The animals were divided into 4 groups. In group I 40 rats were injected, 20 with pindolol and 20 with propranolol, while in group II 24 rats received vasopressin. In group III 56 rats were treated simultaneously half with ADH and pindolol and half with ADH and propranolol. In group IV 38 animals were used as controls; they were treated similarly to group III and subdivided in 2 subgroups of 20 and 18 animals respectively. In the first subgroup the appropriate quantity of distilled water was injected instead of β-receptor blocking agents, while in the second equivalent amounts of distilled water were injected instead of ADH and the β-receptor antagonists.

Results. In 8 of group I animals injected with pindolol and 11 with propranolol there appeared slight degenerative changes of the epithelial tubule cells and slight hyperaemia and haemorrhages of limited extension in the cortex and in the border of cortex and medulla. In group II animals treated by vasopressin there appeared changes consisting of moderate to severe degenerative alterations of cytoplasm and shrinkage of nuclei of the cells of the convoluted tubules, especially in their lower part, and a few thrombotic or thrombohaemorrhagic foci localized mainly between cortex and medulla. Tubules were filled with an amorphous material consisting of mixed desquamated cells and a conspicuous albuminous substance. Foci of medullary necrosis were observed in 8 of animals showing more severe damage (Figure 1). The rats of group III treated concomitantly by ADH and β-adrenergic receptor blocking agents did not develop changes like those produced by vasopressin alone. 16 out of 56 rats presented haemorrhages of limited extension, located mainly at the above-mentioned region between cortex and medulla (Figure 2). Mild epithelial degenerative changes and some amorphous material within tubules was almost observed in all animals. Subgroup a) animals of group IV (control) showed lesions which virtually did not differ than those observed in rats treated by ADH alone. Subgroup b) rats did not show kidney structure changes.

Discussion. In the present experiments, ADH induced kidney lesions of ischaemic type of characteristic localization at the region of the outer part of the medulla and cortex like those described previously[4]. The sensitivity of various branches of the renal arteries to vasopressin seems to be selective, and to suggest the part of the medulla playing the major role in the mechanism of the regulation of urine concentration[1]. The fact that vasopressin-induced damage was prevented by both β-adrenergic receptor antagonists could suggest that ADH effect on the circulation of this particular area is very probably influenced by the adrenergic mechanism. This is also supported by the finding that isoproterenol and epinephrine given in overhydrated rats cause antidiuresis[5,6], in contrast to noradrenaline which induces diuresis, inhibiting the antidiuretic effect of isoproterenol and ADH in animals and of vasopressin in man[7].

On the other hand, the antidiuretic action of ADH is also contributed by β-adrenergic activity indirectly through increase of cyclic AMP concentration in the renal medulla[8].

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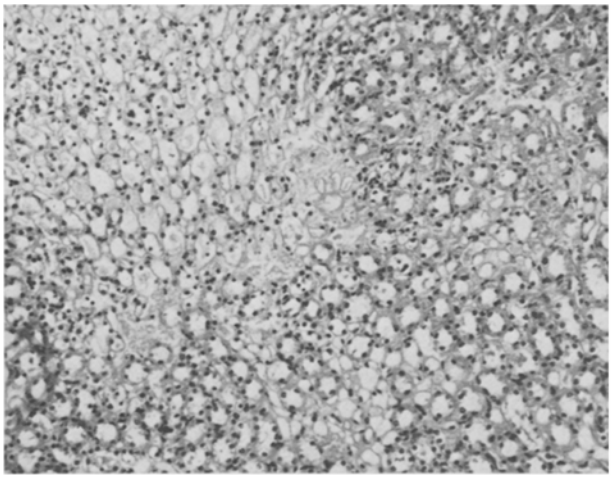

Fig. 1. Kidney of a rat injected once daily for 3 consecutive days with vasopressin i.p. and sacrificed 24 h after the last injection. Foci of necrosis in the lower part of the medulla. ×150, stained with H.E.

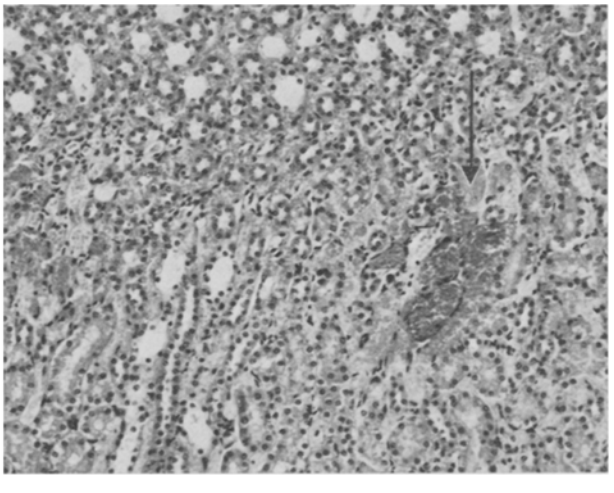

Fig. 2. Kidney of a rat 24 h after combined treatment for 3 days with vasopressin and pindolol. Haemorrhage at the border between cortex and medulla. ×150, stained with H.E.

Conclusions as to the mechanism of the β-adrenoceptive receptor blocking drugs cannot be exactly determined by the present experiments, since there is no evidence concerning the specificity of the effect of pindolol and propranolol. Provided that all β-receptor blocking agents also act as β-receptor stimulating agents, further experiments, such as the effect of low doses of isoproterenol on ADH-induced kidney alterations etc., are needed. The doses used in the present experiments are proportional to those which have used occasionally in man[3]. Independently of the exact mode of action of pindolol and propranolol, their inhibitory effect against ADH could be taken into consideration.

Zusammenfassung. Intraperitoneal injiziertes Vasopressin (einmal täglich während 3 Tagen) führt bei Ratten besonders im Nierenrindenmark-Grenzgebiet zu ischämischen Veränderungen. Pindolol und Propanolol als sogenannte Blockersubstanz, hemmt, bzw, verhindert diese Veränderungen.

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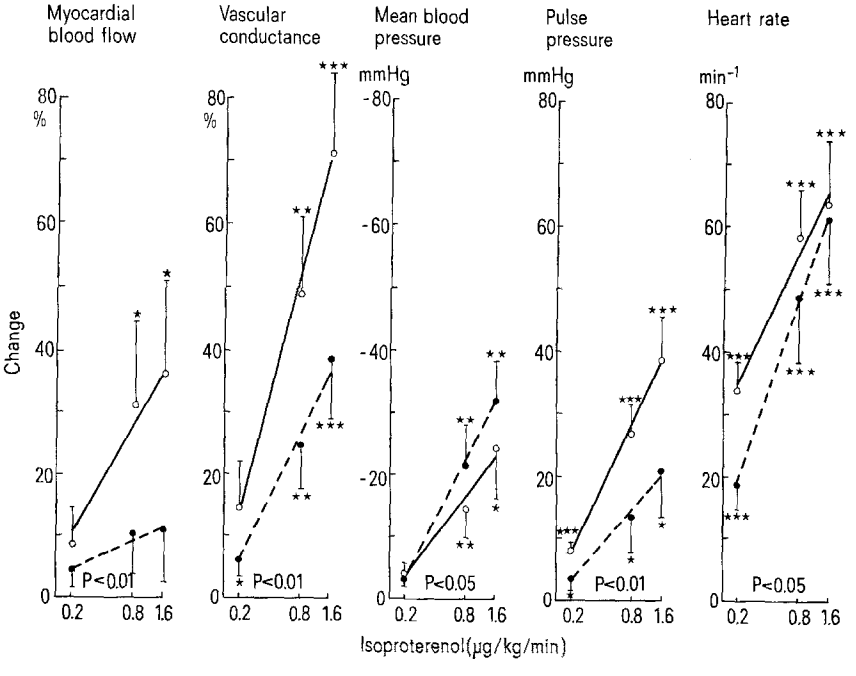
Effect of β-Adrenergic Excitation on Collateral Coronary Blood Flow

Coronary dilatation mediated through β-adrenergic activation may be considered the most important neurohumoral mechanism regulating the blood supply to the normal myocardium. At the same time, little is known of the β-adrenergic effects exerted on the collateral coronary vessels supplying an ischaemic myocardial segment after acute occlusion of its major coronary branch. The present study was designed to elucidate this problem.

Methods. Experiments were performed in 10 open-chest dogs under chloralose (0.11 g/kg body wt.). A myocardial area, amounting to about one-fifth of the total left ventricular mass, was rendered ischaemic by occlusion of the left anterior descending artery (LAD) approximately halfway along its course. Local blood flow of this myocardial segment was measured by means of the heat clearance technique using DC current heated copper-constantan thermal probes. Local flow was continuously registered on a sensitive compensograph. In order to study the collateral flow in a range as wide as possible, the distance of the probe from the supposed centre of the ischaemic zone varied from animal to animal. Local vascular responses to isoproterenol (Isuprel, Winthrop) were registered before and after LAD occlusion, the same myocardial segment serving as its own control. Isoproterenol was infused i.v. in increasing doses of 0.2, 0.8 and 1.6 μg/kg/min. Circulatory parameters were chosen for data analysis during steady state responses. The flow changes were expressed in percentage of the initial control value, by determining zero flow line after having sacrificed the animal. The magnitude of coronary reactions were characterized as local vascular conductance values (%flow/%mean blood pressure) as well, 100% being the normal initial conductance level before LAD occlusion. Thus, vascular changes of a given tissue locus supplied either by the normal or the collateral vessels were defined in the same units. Blood pressure was registered in the carotid artery with the aid of a Statham element; mean pressure was obtained by electrical integration.

Results. LAD occlusion considerably decreased the local myocardial perfusion, while the general circulatory parameters were not modified significantly by this procedure (Table). The effects of β-adrenergic excitation are summed up in the Figure by indicating the linear regression lines of the dose-response relationships. The tissue blood flow of the myocardium increased significantly in the normal state, while it remained fairly constant during the period



Dose-response relationships of the isoproterenol effects. ○—○, normal; ●---●, ischaemic. Vertical bars denote SEM. Symbols refer to the significance of changes from the control values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). P values above the abscisse refer to the significance of differences between the slopes of regression lines.