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Negative inotropic effect of some H_2-receptor antagonists on the isolated human atria[1]

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Summary. H_2-Receptor antagonists were found to possess in various degrees a negative inotropic effect on human atria in vitro. This effect seemed to be independent of H_2-receptor blockade and, at least in the case of oxmetidine, seemed to involve calcium ion transport and/or utilization.

It is well known that histamine possesses a positive inotropic effect on the heart of different species, mediated through excitation of H_2-receptors. Thus, H_2-antagonists could be supposed to possess a negative inotropic effect on the heart. However, in spite of the widespread clinical use of such compounds, reports of any kind of activity on the heart are very few and concern an exceedingly small (less than 0.01%) percentage of subjects[3-5]. This apparent discrepancy may result from the relatively small number of histamine receptors in the heart or the degree of excitation of these receptors which, in physiological conditions, is completely overwhelmed by the excitation of the beta-adrenergic receptors. It is probable that those cases in which some negative effects on the heart following the administration of H_2-blockers were noted were characterized by abnormally high levels of histamine, which contributed consistently to the maintenance of cardiac activity. On the other hand, nonspecific effects of the H_2-antagonists which are known to be present in different tissues, may be responsible for some negative effects of these drugs on the human heart[6]. In the present study, we investigated the action of some new H_2-antagonists of different structure on human isolated heart preparations removed during surgery.

Material and methods. Right atria biopsy samples (1×0.2 cm) obtained during cardiac surgery were used. The technique described by Gristwood et al.[7] was followed. After removal of the tissue it was immediately placed at 4 °C in oxygenated Krebs solution (mM: NaCl 113; $NaHCO_3$ 2.5; KCl 4.7; $CaCl_2$ 1.9; KH_2PO_4 1.2; $MgSO_4$ 1.2; glucose 11.5) and mounted vertically in a 10 ml organ bath at 37 °C. Two platinum electrodes were used to drive electrically the tissue by square wave pulses of 2 msec duration, frequency of 1 Hz and twice threshold voltage. The tissues were allowed to equilibrate at a tension of 1 g for about 60 min. Contractions were recorded by a transducer and a microdynamometer. When the electrically-stimulated contractions were too small, the effect of the compounds was tested against contractions obtained by administering $CaCl_2$ in contractions varying from 0.5 to 3×10^{-3} M.

Drugs. Compounds used were: cimetidine, oxmetidine and compound marked SKF 93479 (kindly supplied by the SKF, Welwyn, England), ranitidine (Glaxo), tiotidine and propranolol (ICI), compound marked DA 4577 4(5)-(4-isopropylaminomethyleniminophenyl)imidazole (De Angeli, Milano, Italy), verapamil (Knoll) and procaine (Fluka).

Results. In our experimental conditions there was no correlation between calcium concentration of the medium and mean contractile force of cardiac biopsy samples. The amount of calcium added to the nutrient fluid was adjusted for each individual preparation in order to obtain a constant contractile response under control conditions.

The mean value of the atrial contraction after calcium administration was 0.85 ± 0.04 g ($n = 35$). In these conditions the effects of the H_2-antagonists varied considerably with the different molecules: cimetidine and ranitidine were virtually ineffective even at the maximum concentration tested (10^{-3} M). At this concentration, tiotidine, which is more potent for its specific effect on the H_2-receptors, showed a remarkable negative inotropic effect. The new compound DA 4577 which again has been shown to be more potent than cimetidine and ranitidine as an H_2-blocker[9], was virtually ineffective in high concentrations (10^{-4} M) and showed a slight negative inotropic effect only at $1\text{-}3 \times 10^{-3}$ M. Conversely oxmetidine and SKF 93479 had a consistent dose-dependent inhibitory effect from

3×10^{-6} to 3×10^{-4} M (fig. 1). The degree of inhibition induced by the different H_2-antagonists was not related to the calcium concentration in the incubation medium.

Other compounds endowed with inhibitory actions were tested for a quantitative comparison with oxmetidine. Verapamil was approximately 30 times as active as oxmetidine, propranolol 10 times as active, and procaine could not be compared because, under our experimental conditions, its response was of the all-or-none type. Oxmetidine and SKF 93479 behaved approximately as verapamil inasmuch as their negative effects could be counteracted by increasing the concentration of calcium ions in the nutrient fluid up to a complete recovery of the preparations. The calcium concentration necessary to counteract the half maximal inhibitory concentration of SKF 93479 and oxmetidine usually corresponded to a concentration twice as high as that used to elicit the initial stimulation of the heart. Figure 2 gives an example of the antagonism of calcium on the inhibitory effect of oxmetidine (6 experiments) and the lack of effect of calcium on the inhibitory effect of procaine (4 experiments). Owing to the limited availability of SKF 93479 only 3 experiments were performed with this compound, which apparently behaved as oxmetidine.

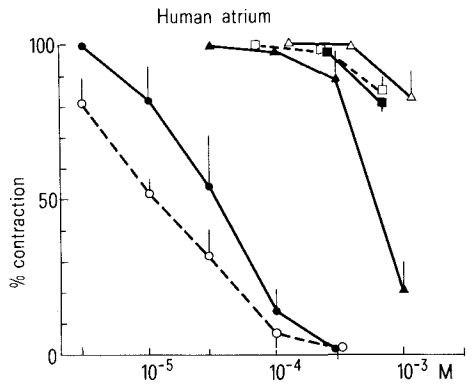

Figure 1. Human atrium strip. On the ordinate percent inhibition of the basal value taken as 100. On the abscissa molar concentrations. ○, SKF 93479; ●, oxmetidine; ▲, tiotidine; ■, DA 4577; □, ranitidine and △, cimetidine. Vertical bars are SE. Each value represents the mean of the values obtained from 4 to 8 experiments.

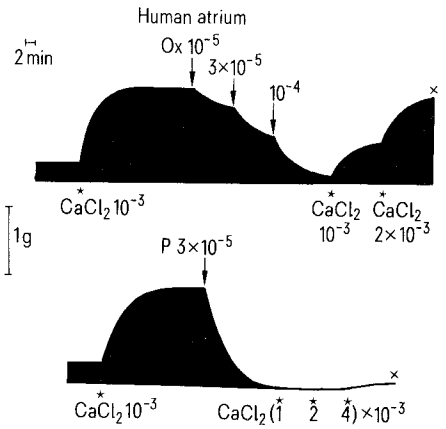

Figure 2. Electrically stimulated human atrium strip. On the ordinate tension of the transducer in grams. Dose-dependent inhibition of the inotropic effect induced by $CaCl_2$, elicited by oxmetidine (Ox) and procaine (P). Doses in molar concentrations. At crosses washing of the preparation.

Discussion. Our data show that the H_2-receptor blockers employed in our experiments possess in various degrees, a negative inotropic effect on isolated human atrium preparations. Cimetidine, ranitidine and DA 4577 were virtually ineffective; they exerted a very slight inhibition of contractile force only in exceptionally high amounts ($1\text{-}3\times10^{-3}$ M). At this concentration, tiotidine, which was inactive at lower concentrations, caused a strong inhibition. Conversely two compounds, namely oxmetidine and SKF 93479, showed a dose-dependent negative inotropic effect in concentrations ranging from 3×10^{-6} M to 3×10^{-4} M. Apparently the effect on the contractile force elicited by the compounds was independent of their ability to inhibit the H_2-receptors. Notably, the range of concentrations used in the present study exceeded by 100–1000 times that necessary to cause a complete H_2-receptor blockade. Oxmetidine was found to have a pA_2 of 7.30 against histamine, with a threshold dose of 10^{-7} M on the guinea-pig papillary muscle[10]; on the guinea-pig isolated atrium, a dose ratio of 2 was obtained at the concentration of 0.2 μM, whereas the same dose-ratio in the rat uterus was obtained at the concentration of 0.056 nM[11]. SKF 93479 was even more potent as an H_2-antagonist with a $pA_2 = 7.78$ in the guinea-pig atrium[8]. Moreover, the order of potency reported in the present study (SKF 93479 > oxmetidine > tiotidine > DA 4577 = ranitidine = cimetidine) was quite different from the order of potency concerning the H_2-receptor blockade on different preparations of the heart or other tissues (tiotidine > SKF 93479 > DA 4577 > oxmetidine > ranitidine > cimetidine)[12–14]. The negative inotropic effect of oxmetidine is unlikely to be connected with a beta-blocking effect since it was shown that this compound does not interfere with adrenergic receptors[11]; moreover in a preceeding investigation[15] we showed, in the rabbit heart, that the negative chronotropic effect of propranolol, a beta-blocking agent, was quite remarkable whereas that of oxmetidine was absolutely negligible. In addition in preliminary experiments[16] we showed in guinea-pig papillary muscle that the inhibition of the isoproterenol effect evoked by oxmetidine is of the non-competitive type with a strong reduction of the maximum response. All of the above observations together with the results of the present investigation suggest that the action of oxmetidine involves an interference with the availability of calcium ions. This hypothesis is also supported by data obtained on the isolated rabbit heart and on gastrointestinal tract[15,17]. Apparently SKF 93479 behaved as oxmetidine but the paucity of the material at our disposal did not allow us to perform an exhaustive investigation of this compound.

Finally DA 4577, found in this study virtually inactive, appeared to be a very interesting substance since it was shown to be a very potent H_2-antagonist (from 3 to 7 times as potent as ranitidine[9]) as an inhibitor of gastric secretion; therefore, the ratio of activity antisecretory effect/negative inotropic effect seems to be extremely favorable to this new H_2-antagonist.

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Intrarenal venous glucose levels in the dog: An evaluation of the sampling technique

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Summary. Analysis of samples of intrarenal venous (IRV) blood from anesthetized dogs demonstrated that IRV glucose concentrations were greater than renal venous and arterial glucose in most samples. However, IRV glucose fluctuated with time such that this technique is unreliable for assessing changes in renal cortical glucose handling during experimental interventions.

Previous attempts to detect renal glucose production in the in vivo canine kidney have been largely unsuccessful since renal venous-arterial (V-A) glucose concentration differences are usually quite small or zero. This is because renal venous drainage reflects both glucose production by the renal cortex and glucose utilization by the medulla[1]. Thus there are directionally opposite processes occurring which tend to negate each other when one attempts to determine glucose differences across the entire organ.

In a previous report[2], we determined that blood samples obtained from the deep veins of the kidney above the corticomedullary junction had plasma glucose concentrations which were usually greater than both arterial and renal venous samples. Therefore, this intrarenal venous drainage apparently has more of a pure cortical component than the mixed drainage into the renal vein.

The present study was undertaken to further evaluate this technique of sampling intrarenal venous blood as a means for studying renal cortical glucose metabolism. More specifically, the question addressed was if the glucose concentration in this drainage remained constant over time.

Methods. Adult mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg). The left femoral artery and vein were cannulated for sampling arterial blood and administering an infusion of isotonic NaCl (2 ml/min), respectively. The left kidney was exposed through a flank incision and the ureter cannulated. A catheter was inserted into the renal vein via the gonadal vein for sampling renal venous blood and an electromagnetic flow probe (Carolina Medical) placed around the renal artery if the latter was not bifurcated. A catheter was then inserted through a stab incision into the renal vein, tied in place with a purse-string suture and advanced up into the deep venous system of the kidney using the criteria of Hinshaw[3] for correct intrarenal venous catheter placement. Arterial pressure and renal blood flow were monitored throughout the experiment with a Grass Model 7D Polygraph.

The experimental protocol consisted of simultaneously taking samples of intrarenal venous, renal venous and arterial blood at 3 min intervals until 10 samples of each were obtained. Thus, the total sampling portion of the experiment took 27 min. All samples were obtained by disconnecting the luer stub adapters from the catheters and letting the blood free-flow into chilled heparin-lithium fluoride treated microfuge tubes, i.e. no syringe withdrawals of blood were used. All tubes were centrifuged with a Beckman Microfuge and plasma glucose determined with a Beckman Glucose Analyzer 2. Urine glucose concentration was also determined and any animal with greater than trace amounts of glucose in the urine was not used for data. At the end of each experiment, the kidney was dissected and checked for correct placement of the intrare-

Glucose concentration differences for intrarenal venous (IRV), renal venous (V) and arterial (A) plasma in the dog (mean ± SE)

Dog	V-A (mmol/l)	p	IRV-A (mmol/l)	p	IRV-V (mmol/l)	p
1	0.03 ± 0.15	n.s.	0.79 ± 0.25	< 0.02	0.76 ± 0.15	< 0.001
2	0.09 ± 0.08	n.s.	0.46 ± 0.06	< 0.001	0.37 ± 0.07	< 0.001
3	0.19 ± 0.07	< 0.05	0.65 ± 0.08	< 0.001	0.46 ± 0.03	< 0.001
4	0.41 ± 0.11	< 0.005	0.82 ± 0.11	< 0.001	0.41 ± 0.07	< 0.001
5	0.58 ± 0.13	< 0.002	0.34 ± 0.17	n.s.	− 0.24 ± 0.09	< 0.05
6	− 0.90 ± 0.09	< 0.001	0.56 ± 0.12	< 0.002	1.46 ± 0.11	< 0.001
7	0.51 ± 0.10	< 0.001	0.36 ± 0.11	< 0.01	− 0.15 ± 0.08	n.s.
8	0.44 ± 0.09	< 0.001	0.16 ± 0.15	n.s.	− 0.28 ± 0.15	n.s.
Mean	0.17 ± 0.06	< 0.005	0.52 ± 0.05	< 0.001	0.35 ± 0.07	< 0.001