Inhibition of the efflux of organic ions from renal cortical slices

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Summary. Cyanine 863 inhibited the efflux of tetraethylammonium ion from slices of rat or rabbit renal cortex while the efflux of p-aminohippuric acid was unaffected. Dinitrophenol increased the efflux rate of both the cation and anion. Cyanine also decreased the dinitrophenol-enhanced efflux of tetraethylammonium (TEA).

The cyanine dye, cyanine 863, referred to hereafter as cyanine, is an inhibitor of the renal transport of organic cations[1,2]. Cyanine has been shown to enhance the nephrotoxicity of the cephalosporin antibiotic cephaloridine in rabbits[3]. Although anion transport is responsible for the entry of cephaloridine into cells of the renal cortex[4,5], cyanine enhancement of cephaloridine nephrotoxicity suggests the possibility that efflux or transport of the zwitterionic cephalosporin out of the proximal tubular cells of the renal cortex may proceed via a cationic transport system. The work of Farah and Frazer[6] demonstrated that cyanine could inhibit the efflux of N-methyl-nicotinamide from slices of dog kidney, although a carrier mediated transport system for organic cations across the luminal membrane has not been further defined.

These in vitro studies were designed to investigate the effect of cyanine on efflux of the classic substrates tetraethylammonium (TEA), a cation, and p-aminohippuric acid (PAH), an anion, from renal cortical slices to provide a foundation for the future study of more complex transport characteristics of zwitterionic molecules.

Methods and Materials. Thin slices of the renal cortex of female adult dutch belted rabbits or female adult wistar rats were prepared with the aid of a Stadie Riggs microtome (A.H. Thomas, Philadelphia, Pa.). 2 (rabbits) or 4 (rats) cortical slices were incubated under oxygen in 3 ml of the buffer described by Cross and Taggart[7] containing 0.01 M sodium acetate (rabbits) or 0.01 M sodium lactate (rats) for 90 min at 25 °C with a shaking rate of 100 cycles per min. ^{14}C-PAH or ^{14}C-TEA, 34.5 μCi/mmole, (New England Nuclear, Boston, Mass.) was added to the incubation medium at 7.4×10^{-5} M. After incubation the slices were rinsed briefly with buffer and transferred to beakers containing 3.0 ml buffer containing no PAH or TEA. The tissue slices were transferred with forceps at 1 min intervals through a total of 30 beakers. Cyanine, 1-ethyl-2-[(1,4-dimethyl-2-phenyl-6-pyrimidinylidine)-methyl] quinolinium chloride (Eastman Kodak Co., Rochester, N.Y.) was added to the efflux buffer at 1.28×10^{-5} M. Dinitrophenol (Eastman Kodak Co.) was added to the efflux buffer at 10^{-4} M. At the end of the 30 min efflux period the tissue and original incubation media were prepared for determination of radioactivity[8] by liquid scintillation counting. Aliquots of the efflux buffer were counted directly. The amount of radiocarbon remaining in the tissue at each minute during efflux was calculated and the rate constant K, for the slow phase of efflux was calculated from the semilogarithmic regression line of the efflux from 6 to 30 min[9].

The effects of the inhibitors added to the efflux medium were studied via paired comparisons in which tissue from a single animal was incubated in 2 beakers containing substrate followed by efflux into either substrate free buffer containing no inhibitor (control) or an identical solution containing either cyanine or DNP. Comparisons were also made between efflux media containing DNP versus DNP plus cyanine. The resulting rate constants were compared using the paired t-test[10].

Results and discussion. Efflux rate constants are presented in the table. The presence of cyanine in the efflux medium

Effect of cyanine and dinitrophenol on efflux of PAH and from TEA pre-loaded renal cortical slices

PAH efflux Comparison	Number	$K(min^{-1})$ $\bar{x} \pm SE$	TEA efflux Comparison	Number	$K(min^{-1})$ $\bar{x} \pm SE$
Rabbit					
Control	3	0.030±0.001	Control	4	0.021±0.003
Cyanine		0.031±0.004	Cyanine		0.016±0.001*
Control	3	0.026±0.004	Control	3	0.022±0.001
No acetate		0.025±0.003	No acetate		0.021±0.001
Control	3	0.030±0.002	Control	3	0.027±0.002
DNP		0.055±0.004*	DNP		0.068±0.004*
DNP	4	0.049±0.006	DNP	3	0.060±0.004
DNP+cyanine		0.043±0.003	DNP+cyanine		0.031±0.002*
Rat					
Control	4	0.041±0.003	Control	4	0.038±0.003
Cyanine		0.039±0.002	Cyanine		0.033±0.002*
Control	3	0.037±0.003	Control	3	0.036±0.001
No lactate		0.036±0.003	No lactate		0.038±0.001
Control	4	0.045±0.004	Control	3	0.038±0.003
DNP		0.067±0.005*	DNP		0.078±0.005*
DNP	6	0.063±0.003	DNP	3	0.076±0.004
DNP+cyanine		0.059±0.003*	DNP+cyanine		0.044±0.007*

*Significantly different at $p \leq 0.05$, paired t-test.

resulted in a significant reduction in the rate of efflux of TEA in both species, but had no effect on PAH runout. The absence of the metabolic substrates acetate or lactate had no effect on the efflux of either the anion or the cation. The addition of DNP, a metabolic inhibitor, markedly enhanced the efflux of PAH and TEA in both the rat and the rabbit compared to control.

The DNP-induced enhancement of efflux correlates with previous observations on the efflux of PAH from dog kidney[9] and supports hypotheses suggesting a highly energy-dependent intracellular accumulation process which, when inhibited, would allow an increase in a more readily diffusable pool available for diffusion (or transport) out of the cell[11,12].

The marked depression of DNP-enhanced efflux of TEA resulting from the further addition of cyanine to the efflux media suggests that transport from the second or 'readily diffusable pool' described above is mediated by a transport system, perhaps at the cell membrane itself, that can be inhibited by cyanine. Cyanine had no effect on PAH efflux from rabbit cortical slices in the presence of DNP and only slightly decreased DNP-enhanced PAH efflux from slices of rat renal cortex. The data emphasize the specificity of the inhibition for organic cation efflux as opposed to a more generalized inhibitory effect. Thus, the cyanine dye, cyanine 863, appears to specifically inhibit the transport of the organic cation, TEA, out of the cells of the renal cortex. Additionally, these studies indicate that the rate of efflux of TEA from rabbit renal cortical slices in the absence of any inhibitor is considerably slower than the efflux of TEA from rat renal cortex or that of PAH in either species (K = 0.021 vs 0.030–0.038).

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Are anticholinergic effects responsible for the heterogeneous quinidine-induced modifications of heart muscle refractoriness?

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Summary. Vagal tone is responsible for the heterogeneous reactivity of atrial and ventricular contractile tissues to quinidine. Acetylcholine may make atrial cells more sensitive to the effects of quinidine.

Over the last 20 years, a dissimilarity in the sensitivity of the different parts of the heart to antiarrhythmic drugs and to quinidine in particular has been shown by means of intracellular recording techniques[2-5]. These results have been confirmed in vivo in our laboratory with experiments on dogs' heart in situ with independent atrial and ventricular activity following the removal of the interventricular septum performed under total cardiopulmonary by-pass: quinidine develops a depressive effect on ventricular ectopic pacemakers, whereas it respects sino-atrial automaticity[6]; it induces a larger increase in the effective refractory period in the atrial contractile tissue than in the ventricular one[7].

With regard to this latter effect on cardiac contractile tissues, the question is whether this dissimilarity is the consequence of quinidine anticholinergic effects[8], since acetylcholine has been shown to be more effective on the atrium[7], or the consequence of an intrinsic disparity in the reactivity of atrial and ventricular contractile fibre due to different electrophysiological properties, since this heterogeneity of response of cardiac contractile tissues to quinidine still exists in dogs with denervated hearts[9].

This present work has been carried out on dogs' heart in situ with independent atrial and ventricular activity, by administering cumulative doses of quinidine after modification of cardiac cholinergic impregnation: either inhibition by atropine, or enhancement by acetylcholine perfusion after bivagotomy.

Methods. The experiments were performed on 6 mongrel dogs for each experimental condition (inhibited and enhanced cardiac cholinergic impregnation) under chloralose anaesthesia (10 ml/kg of a 0.8% solution in saline). In all our experiments, total cardiopulmonary by-pass was performed to remove the interventricular septum and to obtain independent atrial and ventricular activity[6]. With this technique, heart-induced changes in blood pressure are avoided and the recordings are easier to interpret.

The determination of the effective refractory periods (ERP) of atrial and ventricular contractile tissues was made by the extra-stimulus method. The right atrium and the left ventricle were successively stimulated at a basic frequency: period of stimulation 350 msec, by arbitrarily defined suprathreshold (approximate mean threshold, i.e. 1 mA, increased by a factor of 2) square-wave pulses of 5 msec duration delivered by means of 2 myocardial atrial and ventricular unipolar electrodes. The ERP was defined as the shortest interval after the basic stimulus at which an identical extra-stimulus produced a propagated response. Ventricular electrical activity was recorded by means of the derivative of the electrocardiogram (lead I or II) and atrial electrical activity was recorded by means of a direct unipolar electrode fixed on myocardial atrial tissue.

The functional inhibition of cholinergic influence was realized by administration of 0.2 mg/kg of atropine into the extra-corporal circulation circuit. The enhancement of cardiac cholinergic impregnation was realized, after bivagotomy, by a continuous perfusion of acetylcholine (1 mg/kg min) sufficient to produce a significant decrease in atrial frequency ($p < 0.01$) and in atrial contractile tissue ERP ($p < 0.01$), compared to the reference values checked before bivagotomy. Cumulative doses (5, 10 and 20 mg/kg) of quinidine sulphate dissolved in saline were administered after stabilization of the effects of the atropine injection or