Inhibition of $Na^+ + K^+$-ATPase by quaternary ammonium compounds and stimulation of acetylcholine release

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It was previously reported that tetraethylammonium (TEA) and edrophonium competitively inhibited the stimulation of brain $Na^+ + K^+$-ATPase produced by K^+ (Abdul-Cader, Eaves, Wood & Nowell, 1973). (10^{-4} M), edrophonium (10^{-4} M) and DMP (10^{-5} M) significantly increased acetylcholine output from $5.3 \pm 0.5 - 7.9 \pm 0.5$ ng g^{-1} min^{-1} ($P < 0.01$), $5.3 \pm 0.4 - 8.3 \pm 0.4$ ng g^{-1} min^{-1} ($P \ll 0.001$) and $5.9 \pm 0.7 - 8.1 \pm 0.5$ ng g^{-1} min^{-1} ($P < 0.02$) respectively ($\pm$ s.e. mean; $n=6$; paired 't' test); $P=0.03$ by Wilcoxon ranking test. The output returned to the control level when the drugs were washed out. In comparison, ouabain (10^{-5} M) increased the output from $6.2 \pm 0.6 - 18.6 \pm 2.8$ ng g^{-1} min^{-1} ($\pm$ s.e. mean; $n=6$) and it remained at approximately this level for 10 min after washing out.

Acetylcholine output generally became irregular with occasional high discharges when the slices were exposed for longer periods to TEA, edrophonium,

Table 1 Comparison of the inhibitory effects of quaternary ammonium impounds on $Na^+ + K^+$-ATPase activity of Lubrol-extracted enzyme from guinea-pig brain

		% Inhibition of $Na^+ + K^+$-ATPase activity		
Drug	*Concentration (mole/litre)*	*Maximally stimulated (110 mM NaCl/ 10 mM KCl)*	*Half-maximally stimulated by K^+ (110 mM NaCl/ 1 mM KCl)*	*Half-maximally stimulated by Na^+ (20 mM NaCl/ 10 mM KCl)*
TEA Cl	10^{-1}	44.1	85.0	26.0
	10^{-2}	7.5	24.0	NSE
Edrophonium Cl	10^{-2}	33.9	85.0	68.0
	10^{-3}	4.1	32.0	11.0
Bretylium tosylate	10^{-3}	6.5	46.3	5.6
	10^{-4}	7.0	13.4	NSE
TBA Cl	10^{-3}	22.7	51.0	7.0
	10^{-4}	NSE	17.2	NSE
HC_3Br	10^{-4}*	NSE	26.9	NSE
DMP Cl	2.5×10^{-4}*	30.1	42.0	14.2
	10^{-4}	13.2	20.0	NSE
TM10 Br	10^{-3}	14.9	66.0	29.2
	10^{-4}	NSE	29.8	9.4

Experimental details as described by Shirachi, Allard & Trevor (1970) and Abdul-Cader, Eaves, Wood & Nowell (1973). Results expressed as means of triplicate determinations. NSE = no significant effect.
* Higher concentrations could not be used due to interference with the colorimetic method.

Further work suggested that bretylium, tetrabutylammonium (TBA), hemicholinium (HC_3), dimethylquaternary propranolol (DMP) and xylocholine (TM10) were also competitive against K^+ since they inhibited the enzyme system more effectively at half-maximal K^+ concentration like TEA and edrophonium (Table 1).

In view of reports that conditions leading to inhibition of $Na^+ + K^+$-ATPase enhance acetylcholine release (Paton, Vizi & Zar, 1971; Vizi, 1972), the above drugs were tested for effects on acetylcholine output from rat brain cortical slices using the method of Vizi (1972) with minor modifications. Acetylcholine output during exposure to the drug for 10 min was compared with the mean control output for the same slices during the previous 20 minutes. TEA bretylium, TBA, DMP and TM10 in the 10^{-5}–10^{-4} M concentration range and HC_3 (10^{-4} M), whereas HC_3 (10^{-5}M) appeared to decrease output. Lowering the K^+ concentration to 1.9 mM also produced erratic effects with a high average output of 12.7 ± 0.8 ng g^{-1} min^{-1} ($\pm$ s.e. mean; $n=9$) over 60 min and in one of these experiments exposure to edrophonium (10^{-5} M) for 30 min resulted in an output of approximately 1 μg g^{-1} min^{-1} pver a 10 min period.

Work is currently in progress to explore the possibility which arises that quaternary ammonium compounds may enhance acetylcholine release by inhibiting the stimulation of $Na^+ + K^+$-ATPase produced by K^+.

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Detection of small amounts of prostaglandin (PG)-like material and rabbit aorta contracting substance (RCS) released into the blood of the rat

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The cascade superfusion technique (Finkleman, 1930; Vane, 1964) is widely used for the detection and bioassay of biologically active substances. The method has also been employed to detect the release of vasoactive substances into the circulation of anaesthetized animals (Vane, 1964, 1969). However, because of the relatively high flow-rates required, the blood-bathed organ technique is very difficult to use with small animals.

Recently, Ferreira & de Souza Costa (1976) have described a laminar flow superfusion technique which can be used to detect very small amounts of biologically active material with very slow rates of superfusion and this latter apparatus has now been adapted for the blood-bathed organ technique in rats, as suggested by Ferreira & de Souza Costa (1976).

The apparatus is shown in Figure 1. Blood is removed at 0.1 ml/min through a polyethylene cannula in a carotid artery and pumped directly over a rabbit aorta and rat stomach strip, set up in a way to be described elsewhere, Krebs (gassed with 95% O_2 + 5% CO_2) containing antagonists (Gilmore, Vane & Wyllie, 1968), is simultaneously passed over the tissues. This Krebs flow is initially set at 0.2 ml/min, then reduced to 0.1 ml/min as the blood reaches the tissues, in order to maintain a constant flow over the tissues. Standard solutions are injected directly over the tissues and plasma volume is maintained by i.v. injection of 6% dextran/saline until the animal dies (up to 1 hour).

Using this technique we have detected the release of

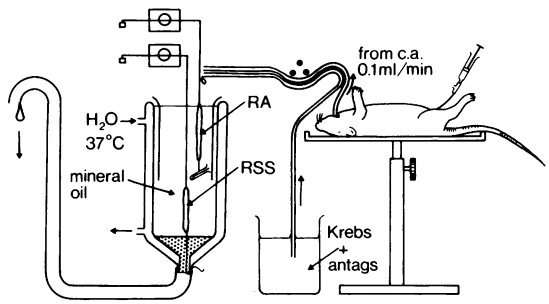

Figure 1 Blood-bathed organ technique in the rat. All tubing is kept as short as possible to reduce temperature changes and the rat is moved as close as possible to the organ bath, using an adjustable operating table. RA = rabbit aorta, RSS = rat stomach strip, c.a. = carotid artery.

RCS and PG-like material into the blood, following i.v. injection (into femoral or tail vein) of 0.5 mg/kg of pig pancreatic phospholipase A_2. With the use of appropriate tissues, it should also be possible to detect the release of other biologically active substances into the circulation of the rat.

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