A time series experimental design[7] was used (multiple unit, single intervention) with a pre- and post-intervention period. Experimental observations were made for 120 min, divided into 3 40-min periods. During the pre-meditation or pre-relaxation period (0–40 min), subjects sat with eyes open. Subsequently (40–80 min), they were instructed to close their eyes and start meditation, or in the case of controls, simply to rest or relax. During the post-meditation or post-relaxation period (80–120 min), they continued to sit but with eyes open again. Since drowsiness (EEG stage 1) or sleep (EEG stages 2, 3, 4) may occur during TM[8], we monitored a unipolar electroencephalogram (EEG), electromyogram (EMG), and electro-oculogram (EOG) according to standard methods[9]. Questionnaires were administered in which subjects judged the quality and normalcy of meditation or rest periods after the experiment.

Prior to beginning measurements, an arterial catheter was placed p.c. into a brachial or radial artery. Blood samples were taken every 20 min (2 samples in each of the 40-min periods). Plasma cortisol was measured by the competitive protein binding method of Murphy et al.[10]. For analysis of the trend of cortisol concentration, curvilinear regression analysis of variation over time with a test of significance of the coefficients was used in statistical treatment of the data[11,12].

Results and discussion. Trend of cortisol concentration (mean ±SE) over course of the experiment in controls, restudied controls, and long-term practitioners is shown in the figure. Pre-relaxation and pre-meditation initial values (μg%; mean±SE) at 10 and 30 min for controls were: 5.5±0.5, 5.4±0.6; for restudied controls: 5.8±0.5, 5.1±0.5; for long-term practitioners: 5.7±0.6, 5.4±0.8. Cortisol values in long-term practitioners declined rapidly (27%) during meditation ($p < 0.01$; significance of regression). The decline of cortisol concentration approximated 1.5 mg/100 ml. The trend of mean cortisol concentration in long-term practitioners differed significantly ($p < 0.03$) from that of the control group. Subjects reported comfortable, usual TM or relaxation experience during the measurements.

On the average we found that rest and TM periods were associated with almost identical amounts of sleep: 70% of meditation or rest time was spent in wakefulness, 22% in stage 1 and 8% in stages 2 and 3. There was no correlation between total sleep time and cortisol decline and therefore sleep alone cannot account for the decline observed. Also, TM differs from sleep in this effect since sleep is not correlated with acute alteration of plasma cortisol[13]. These conclusions do not support the hypothesis of Pagano et al.[8] that sleep during meditation is responsible for its beneficial effects.

Since a close qualitative relationship between ACTH and cortisol secretory episodes exists in normal individuals[14], it seems likely that the acute decline of plasma cortisol reflects lessened pituitary-adrenal activation and not a non-specific increase in the metabolic clearance of cortisol; this is further supported by the fact that blood flow to the liver, the principal site of cortisol degradation, actually declines markedly during TM[4]. Based upon the assumption of a 70-min half life for circulating cortisol[6,14], a 27% decline of cortisol concentration in 30 min is consistent with complete inhibition of pituitary-adrenal activity. If no secretion of cortisol occurred for 30 min, plasma levels would decrease to $C_o e^{(\ln 0.5) \times 30/70} = C_o \times (0.74)$; i.e., a 26% decrease.

This report is the 1st documentation of self-induced decrease of adrenocortical activity; whether stylized practices of rest or relaxation-induction other than TM would have a similar effect is a matter of conjecture.

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Electrogenic hyperpolarization in canine cardiac Purkinje fibres exposed to calcium ionophores[1]

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Summary. The Ca ionophores markedly enhance the increase of intracellular Ca occurring during Na-free perfusion and the hyperpolarization observed upon Na readmission may be due to rapid restoration of intracellular Na and resultant stimulation of both electrogenic sodium and calcium efflux.

Ca efflux from cardiac muscle is dependent upon external Na and Ca[2,3]. When intracellular calcium concentration (Ca_i) is increased, Na-dependent Ca-efflux increases[4]. X-537A and other Ca ionophores which increase Ca_i cause hyperpolarization of the membrane potential in canine cardiac Purkinje fibres (CPF)[5,6]. The present study was undertaken to determine whether this effect of X-537A was dependent upon the presence of Na during exposure to the ionophore.

Methods. Details of the tissue bath and experimental set-up

have been described elsewhere[5,6]. After control records were obtained in oxygenated solution (36 °C) of the following composition (mM/l): Na = 150.8; Ca = 2.7; K = 4.0; Mg = 0.5; Cl = 147.4; HCO_3 = 12.0; glucose = 5.5; HPO_4 = 1.8, using standard microelectrode techniques to measure the membrane voltage of excised CPF, perfusion with oxygenated (100% O_2) Na-free solution of the following composition (mM/l) was begun: Choline = 148.3; Ca = 2.7; K = 4.0; Mg = 0.5; Cl = 158.7; glucose = 5.5. The Na-free solution was buffered to a pH = 7.4 by balancing Trizma Base with 0.1 N HCl.

In 3 preparations, the 'control' experiment consisted of exposing the CPF to Na-free solution for 45 min without added ionophore (stimulus externally applied at a rate of 1.67 Hz). The action potential stopped within 5 min of beginning perfusion with Na-free solution. Na-containing solution was readmitted to the experimental preparation after 60 min and the CPF was permitted to regain control levels of action potential parameters (amplitude of the overshoot and plateau, and diastolic potential).

The drug experiment consisted of exposing the same CPF to a 2nd period of Na-free perfusion for 30 min and then adding Ca ionophore, X-537A, 10^{-5} M, to the Na-free perfusing solution for an additional 30 min. Sodium-containing solution without ionophore was then restarted and action potential parameters were recorded during a wash-out period of about 60 min. On 3 occasions there were no 'control' experiments prior to exposure to Na-free solution containing the ionophore. In one of these 3 experiments, A23187, 10^{-5} M, an ionophore more specific for Ca than is X-537A, was used[7].

Results. The figure shows action potentials from a typical experiment. The control action potential is shown in the figure, A. The maximum diastolic potential (MDP) is − 90 mV. Following the control period Na-free perfusion was maintained for 60 min. The figure, B, shows the action potential 6 min after readmitting Na to the Na-depleted fibre. MDP has increased to − 93 mV and action potential duration is prolonged. (Prolongation of action potential duration shown in the figure, B, may be the result of prolonged exposure to the quaternary ammonium ion, choline[8].) The figure, C, shows the action potential 65 min after readmitting Na. MDP and overshoot of the rapid upstroke have returned to control values. Action potential duration and plateau duration are still prolonged as compared to control (figure, A). Na-free solution was readmitted and, after 30 min, X-537A (10^{-5} M) was added to the Na-free perfusate. Exposure to Na-free/X537A solution lasted an additional 30 min. The figure, D, shows the action potential 4 min after restoring control solution. MDP has increased to − 101 mV. Action potential duration (measured from the onset upstroke of the action potential to 100% repolarization) is decreased to 65% of the value during the initial control period. (The effect of Ca-ionophore exposure on action potential duration (figure, D), the opposite of the effect seen in the figure, B, during the control experiment, is probably due to the ionophore-enhanced increase in Ca_i and is not due to the greater degree of hyperpolarization following ionophore exposure[9].) Figure 1, E shows the action potential after 60 min wash-out with control solution. The plateau duration and amplitude, and action potential duration have not fully returned to control levels while diastolic potential has returned to control levels (figure, A).

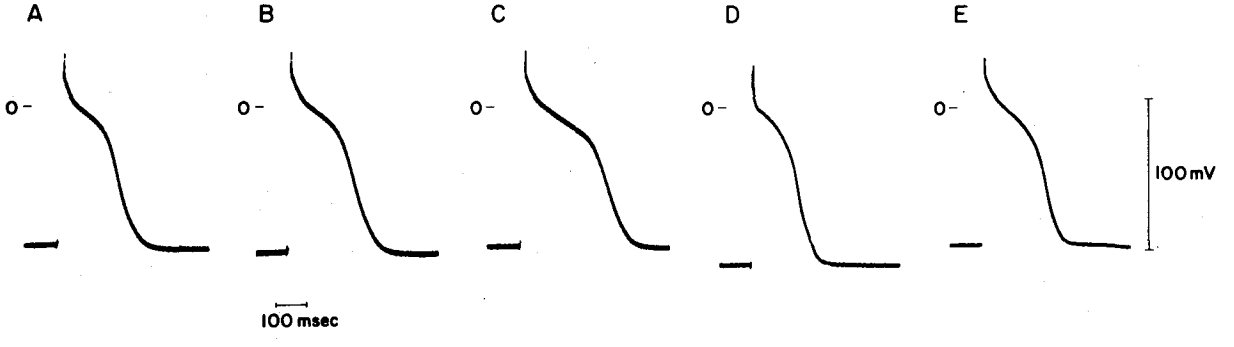
The maximum negative membrane voltage recorded following Na readmission to the 6 preparations during the drug experiments were (time of the recording following Na readmission is in parentheses): − 101 mV (4 min); − 102 mV (7 min); − 98 mV (10 min); − 102 mV (10 min); − 95 mV (6 min); − 96 mV (8 min). The 1st 2 values are more negative than − 100 mV, the E_K when K_o = 4 mM[10]. The mean magnitude of hyperpolarization in the 3 control experiments was − 3.7 mV. In 5 experiments with X-537A and in 1 experiment using A23187, the mean magnitude of hyperpolarization was − 8.9 mV. The results of the 6 experiments were analyzed with a 2-tailed student's t-test and found to be statistically significant with a $p < 0.025$.

Discussion. X537A transports Na, Ca, and other cations across cell membranes in the direction of their concentration gradient[11]. We have previously shown that X537A and A23187 have effects on CPF that are best interpreted as being due to their ability to increase Ca_i[5,6]. In the present experiments, the effects of the ionophores on the action potential do not seem to depend on the presence of Na_o during ionophore exposure. Although the relation between the Na-free/ionophore-induced increase in Ca_i and membrane hyperpolarization remains uncertain, the observation suggests several possible explanations: 1. increased membrane resistance; 2. decreased inward current; 3. enhanced activity of electrogenic Ca efflux; 4. enhanced activity of an electrogenic Na pump; 5. increased K conductance.

1. Ionophore exposure during perfusion with Na-containing Tyrode solution causes the membrane potential to hyperpolarize and causes the slope (dV/dT) of diastolic depolarization to decrease[6], 2 effects which are usually the result of decreased membrane resistance (increased K permeability) resulting in closer approximation of the membrane potential to E_K.

2. Inward Ca current probably contributes little to membrane current-voltage relations at potentials negative to − 50 mV[12] and Na inward current increases during Na readmission.

3. Marshall and Kroeger[13] suggested that electrogenic Ca extrusion is the cause of adrenergic hyperpolarization in



Effects of sodium-free perfusion on the action potential of a canine cardiac Purkinje fibre before (A and B) and after (C and D) ionophore exposure, E, washout. See text for full description.

uterine smooth muscle. Calculations similar to those performed by Marshall and Kroeger[13] based on the findings of -8.9 mV hyperpolarization in these experiments would give a Ca efflux of 0.7×10^{-9} m Ca min^{-1} cm^{-2}. It is therefore possible that electrogenic Ca efflux can account for the hyperpolarization observed[14].

4. During the 1st few min following readmission of Na, rapid replenishment of Na_i occurs enhanced by the abundant amount of Ca_i available for exchange[2,13]. Thus, within min there may be marked enhancement of electrogenic Na-K exchange mediated by the Na-K ATPase[15]. Increased Ca_i may also stimulate the Na pump.

5. Ca_i is thought to modulate K permeability in many excitable cells and increasing Ca_i might increase K permeability in CPF[16]. However, a change in K permeability is probably not the sole cause of the hyperpolarization observed because a) this effect should be observed during Na-free perfusion whereas hyperpolarization invariably occurred 4–10 min after Na-readmission; and b) K probably accumulates in the intracellular clefts during Na-free perfusion resulting in a transient decrease of the K-equilibrium potential[10].

The hyperpolarization following Na-readmission to ionophore-treated fibres is probably both a result of enhanced electrogenic Na efflux that is related to the ionophore-mediated increase in Ca_i and electrogenic Ca efflux. Preliminary experiments with readmission of Li-substituted solution and in fibres exposed to acetyl-strophanthidin during ionophore-exposure support this suggestion.

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Localization of renal kallikrein in the dog

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Summary. Renal kallikrein was estimated in glomerular, tubular and medullary fractions of dog kidneys. It was found primarily in the cortex, the highest level of activity being detected in a glomeruli-rich fraction. These results support previous observation that kallikrein may be associated with the juxta-glomerular complex.

Renal kallikrein is probably the source of kallikrein found in the urine because the 2 enzymes are indistinguishable, while both differ from the kallikreins of plasma[1–3]. Renal kallikrein has been localized primarily to the cortex of the rat[4] and dog kidney[5–7], the highest concentration being found in the outer cortex[7]. Early studies by indirect methods indicated localization within the proximal tubule[4,8], however, more recently, utilizing stop-flow techniques in the dog[9] and fluorescent antibody histochemistry in the rat[10], kallikrein has been localized to the distal tubule and possibly the macula densa of the juxtaglomerular apparatus.

The objective of this study was to examine the distribution of kallikrein in glomerular and tubular fractions derived from dog renal cortex. Studies with rat kidney indicate that, when compared with tubular tissue, glomerular kallikrein concentration may be relatively high[11]; however, in another study glomeruli devoid of juxtaglomerular apparatus accounted for only 15% of the activity of the total cortex[7].

Methods. Dogs (8–12 kg mongrel) were anaesthetised with sodium pentobarbital (20 mg kg^{-1} i.v.) and the kidneys perfused in situ with sucrose (0.25 moles l^{-1}). The method described by Cook and Pickering[12] was then used to separate a glomerular fraction from the tubular tissues of the cortex. 3 fractions were obtained; cortical tissue rich in glomeruli, cortical tissue poor in glomeruli (mainly tubules) and medulla. An aliquot of each sample was taken for histological observation and the remaining tissue lyophilised. The lyophilised tissue was homogenised in distilled water (25–50 mg ml^{-1}) followed by centrifuging (10,000 × g, 4 °C, 20 min). The supernatant was acidified to pH 2.0–2.5 with HCl (0.1 moles l^{-1}) for 20 min at room temperature (to inhibit kininase). It was then adjusted to pH 9.0 with NaOH (0.1 moles l^{-1}) and centrifuged. Aliquots of the supernatant (0.05–0.4 ml) were incubated at 37 °C for 20 min with 15 mg dog kininogen (modified from Rocha E. Silva et al.[13]) 50 mg ml^{-1} in bicarbonate buffer[14] at pH 9.0. Incubates were applied to an isolated guinea-pig ileum preparation suspended in magnesium-free Tyrode solution containing atropine and mepyramine (both 2×10^{-8} g ml^{-1}) aerated with 95% O_2, 5% CO_2. Kinin present in the incubates was determined by comparison with doses of synthetic bradykinin (BRS 640, Sandoz) by 6 point bio-assay. The kallikrein content of an extract was expressed as the amount of synthetic bradykinin equivalent in potency to the kinin liberated by the extract per mg of protein during 20 min incubation. Protein was measured by the method of Lowry et al.[15].

Qualitative tests. To exclude the possibility of contamination by smooth muscle stimulants, the extracts and kininogen were incubated separately at pH 9.0 for 20 min with an equal volume of 0.16 moles l^{-1} NaCl, and the incubates