Systolic blood pressure (BP) and serum urea (BUN) and creatinine (Cr) concentrations in uninephrectomized rats before and after renal artery clipping

Groups	Before clipping			28 Days after clipping		
	BP (mmHg)	BUN (mg/dl)	Cr (mg/dl)	BP (mmHg)	BUN (mg/dl)	Cr (mg/dl)
I	125 ± 3[a]	13.8 ± 0.1	0.55 ± 0.04	173 ± 4[c]	23.5 ± 0.4[c]	1.00 ± 0.06[c]
II	125 ± 2	16.6 ± 0.1[b]	0.69 ± 0.04[b]	152 ± 2[b, c]	24.1 ± 0.9[c]	1.05 ± 0.07[c]
III	121 ± 3	17.3 ± 1.0[b]	0.70 ± 0.05[b]	172 ± 4[c]	25.4 ± 2.5[c]	1.14 ± 0.08[c]

10 animals in each group. [a] Mean ± 1 SE. [b] $p < 0.05$ when compared to group I. [c] $p < 0.01$ when compared to the basal value for the same group.

for another 17 days. At the end of the experiments, plasma urea and creatinine concentrations were determined in 10 animals from each group, all rats were killed, and the transplants were removed and examined as previously described[10]. All animals included in the study met the following criteria: 1. histological evidence of successful transplantation; and 2. systolic blood pressure at the beginning of the study below 140 mm Hg. The criterion for hypertension was BP > 149 mm Hg (3 SD above the mean blood pressure of 598 animals of this strain). Results are expressed as mean ± 1 SE.

Results and discussion. No significant difference in the average body weight was observed between the groups at any time. Serum urea and creatinine concentrations are shown in the Table. The characteristics of the autotransplants of both renal medulla and cortex were consistent with those described earlier[10]. The morphological features of the renomedullary transplants in the present case were consistent with those shown to protect against hypertension[9, 10].

The results of the present study (Figure 1) have shown that transplantation of the renal medulla, but not of renal cortex, abolishes the blood pressure rise in the course of renal hypertension. Moreover, after transplant removal, the blood pressure in group II rose within 1 week to the level found in groups I and III (Figure 2), a finding that further suggests antihypertensive actions by renomedullary tissue. The presented data are in close agreement with the previous reports on the antihypertensive effect of renomedullary transplants in one-kidney renal hypertension[8].

The finding of particular interest was that after renal artery clipping the time course of blood pressure increase and the final level of blood pressure reached were similar in rats from groups I (with normal medulla inside the clipped kidney) and III (with almost completely atrophied medulla). However, the presence of medullary tissue outside of the clipped kidney (group II) protected the animals against hypertension. These results suggest that renal artery clipping interferes in some way with the antihypertensive function of the renal medulla. Thus, the development of renal hypertension apparently involves clip-induced renomedullary deficiency as well as the known roles of the renin-angiotensin system and sodium and water retention[15, 16]. In quantitative terms this contribution may be on the order of 20–30 mm Hg, thus representing about one half of the total blood pressure rise in the present case.

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Sodium Pump: its Importance to Intercellular Communication in Heart Fibres[1]

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Summary. The effect of ouabain on the electrical coupling of canine Purkinje cells was investigated. It was found that the glycoside decreases cell communication through an increase in junctional resistance, what supports the view[2] that the sodium pump has an important role on the control of cell communication.

Previous observations from our laboratory[2, 3] have shown that the injection of sodium ions into a heart or liver cell (De Mello, unpublished) produces electrical uncoupling. These observations are probably explained by the increment of the intracellular calcium concentration which follows the raise of the intracellular sodium content[4, 5]. Evidence has been presented that calcium is, indeed, involved in the control of junctional permeability in epithelia[6] and in cardiac Purkinje fibers[7, 8]. In support of this idea is our recent finding that the injection of sodium ions into a cardiac Purkinje cell, immersed in low calcium solution, had a small effect on cell communica-

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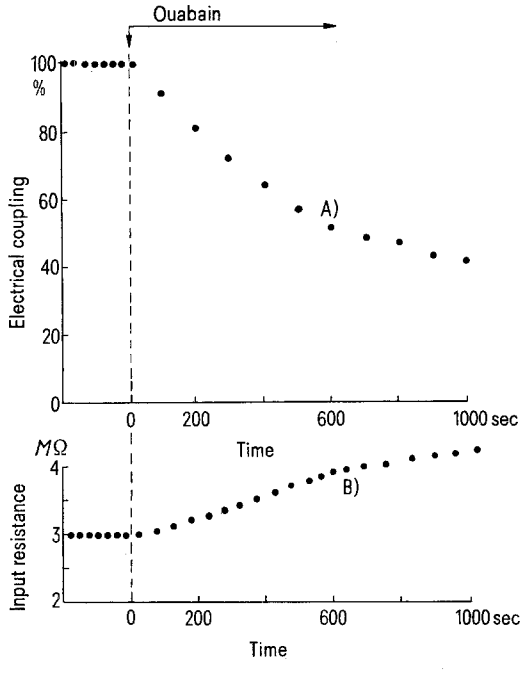

Fig. 1. Effect of ouabain on the electrical coupling of Purkinje cells. A) Decrease of intercellular communication caused by ouabain (6.8×10^{-7} *M*) (average from 3 experiments). B) Increase of input resistance recorded from a single Purkinje cell during the action of the glycoside (average from 3 experiments). Temperature 36 °C.

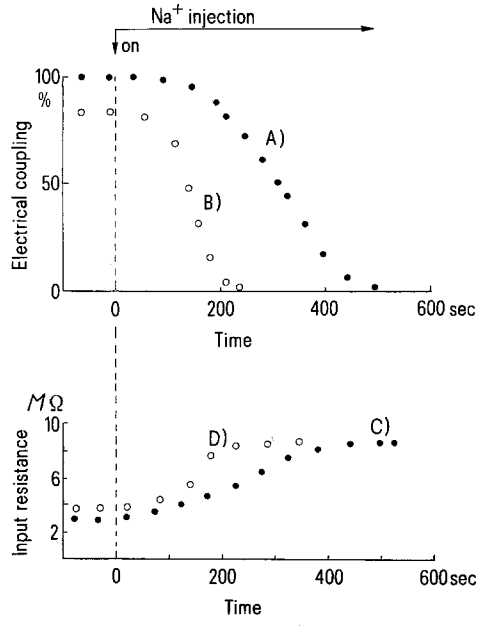

Fig. 2. Influence of ouabain on the rate of cell uncoupling produced by intracellular sodium injection. A) Suppression of cell communication caused by the injection of sodium ions into a normal Purkinje cell (average from 3 experiments). B) Shows the action of ouabain (6.8×10^{-7} *M*) accelerating the uncoupling produced by intracellular sodium injection. The treatment with ouabain started 5 min before sodium injection (average from 3 experiments). C) and D) Show increase of input resistance of the injected cell recorded simultaneously with cell communication before (C) and after treatment with ouabain (D) (average from 3 experiments). Temperature 36 °C.

tion. The restablishment of the external calcium concentration (2.7 m*M*) after 400–500 sec of sodium injection, lead immediately to cell uncoupling (DE MELLO, unpublished).

The suppression of cell communication produced by intracellular sodium injection is highly indicative that the sodium pump is largely involved in the control of intercellular communication. Preliminary studies[9] indicated, indeed, that the inhibition of the sodium pump in canine Purkinje fibres causes electrical uncoupling. This possibility was further investigated in the present work.

Experiments were made on isolated strands of canine Purkinje fibres. The animals were anesthetized with sodium pentobarbital i.v. and the heart immediately removed and immersed in cooled Tyrode's solution to reduce the oxygen debt. Strands of Purkinje fibres were then dissected from the left ventricle and transferred to a transparent chamber through which Tyrode's solution with the following composition flowed continuously (m*M*): NaCl – 137; KCl – 5.4; $CaCl_2$ – 2.7; $NaHCO_3$ – 12; $MgCl_2$ – 0.5; NaH_2PO_4 – 3.6 and dextrose – 5.5. The saline solution was saturated with a mixture of 5% CO_2 and 95% O_2 and kept at 36 °C. pH of solution was 7.

Ouabain (Sigma Chemical Co.) that inhibits the sodium pump in therapeutic doses[10] was used at a concentration of 6.8×10^{-7} *M*. Microelectrodes (10–15 *MΩ*) filled with NaCl (2 *M*) were used to inject the ion into the cell according to the techniques of NASTUK[11] and DEL CASTILLO and KATZ[12]. The injection of sodium was made applying outward rectangular current pulses (60 msec in duration, 6 c/s) delivered by an electronic stimulator and isolation unit to the interior of the micropipette. The electrical coupling was measured by injecting current pulses into a Purkinje cell through one microelectrode and recording the resulting voltage change with another microelectrode impaled in an adjacent cell[13]. Conventional 3 *M* KCl microelectrodes (10–15 *MΩ* resistance) were connected to a standard cathode follower and d.c. amplifier. The voltage changes as well as the current pulses were displayed simultaneously in a 565 Tektronix oscilloscope and pen recorder (Dynograph). The input resistance of Purkinje cells was measured with a single microelectrode connected to a balance bridge circuit serving to pass current pulses and record voltage changes. Stimulation was made with a pair of fine silver electrodes gently applied to the surface of the fibre.

The results obtained indicated that cell communication is largely impaired by ouabain. As can be seen in Figure 1 A (average from 3 experiments) the electrical coupling of adjacent Purkinje cells was gradually reduced by the glycoside (6.8×10^{-7} *M*) reaching 50% of the control value in 10 min of incubation. The input resistance of single Purkinje cells was increased concomitantly with the fall in cell communication (see Figure 1 B) what means that the decrease in size of the electrotonic potentials was not related to a reduction in resistance of the nonjunctional cell membrane. On the other hand, the increase in input resistance was not related to depolarization of the surface cell membrane. The resisting potential was slightly reduced (about 4 mV) during the first 25 min. This is certainly related to the use of an increased extracellular potassium concentration (5.4 m*M*) which is known to decrease ouabain toxicity[14].

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Preliminary experiments performed on mammalian liver cells (guinea-pig) showed similar results, that is, ouabain (6.8×10^{-7} *M*) reduced cell communication by 60% in about 10 min.

Uncoupling of heart cells produced by intracellular sodium injection was achieved more rapidly in presence of ouabain. As is illustrated in Figure 2A (average from 3 experiments) the injection of sodium into a Purkinje cell abolished the electrical coupling in 500 sec. Experiments performed on the same fibres exposed to ouabain (6.8×10^{-7} *M*) showed that the intracellular sodium injection caused uncoupling in 230 sec (see Figure 2B – average from 3 experiments). These results are probably explained by the build up of a larger intracellular sodium concentration in a shorter period of time since the extrusion of sodium was reduced or abolished by ouabain. In both situations the input resistance of the injected cell was increased as is shown in Figure 2, C and D.

Stimulation of the heart fibres at a high rate (3 c/s) that is known to raise the intracellular sodium concentration also lead to accentuated decrease of cell communication in Purkinje fibres exposed to ouabain. On these experiments, the hyperpolarization usually elicited by stimulation at a high rate in normal fibres[15] was negligible or absent, and in some experiments a depolarization was found, what probably contributed to the reduction of cell communication by increasing the inward movement of calcium.

In summary, the results presented above indicate that the sodium pump plays an important role on the regulation of cell communication in heart muscle. These observations highly suggest that the block of impulse conduction caused by cardiac glycosides in cardiac tissues, can be due, at least in part, to an increase in junctional resistance. The mechanism by which ouabain impairs cell communication in Purkinje fibres is probably related to the increase of the intracellular calcium content that follows the increment of the internal sodium concentration. A similar effect of ouabain in ventricular muscle has recently been reported by WEINGART[16]. The fall of intercellular communication found in liver cells exposed to the glycoside seems to indicate that the role of sodium extrusion on maintaining a high conductance pathway between cells is not limited to cardiac muscle.

An important implication of these results is that the intercellular movement of ions and molecules[17] can be largely reduced or abolished by suppression of the sodium pump. The physiological and pathological meaning of these observations are obvious and requires further investigation.

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Behavior and Endocrine Effects of 3,4,5-Trimethoxyamphetamine in Male Mice

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Summary. Effects of single doses of 50 and 100 mg/kg of TMA given i.p. were noted in male albino mice after 40 min and $2^1/_2$ h. Locomotor activity was significantly altered and biochemical tests indicated stimulatory effects on adrenocortical and adrenomedullary functions due to TMA.

In previous studies SACKLER et al. have investigated behavioral and endocrine effects of LSD-25[1,2], and mescaline[3,4] in laboratory animals in expectation that pertinent data could be obtained concerning possible biochemical basis in the etiology and pathophysiology of schizophrenias. PERETZ et al.[5] first reported that a derivative of amphetamine namely 3,4,5-trimethoxyamphetamine (TMA) was also hallucinogenic in man producing effects similar to its close chemical, psychomimetic relative mescaline. Chronic intake of amphetamine has similarly been known to cause paranoid psychoses in certain addicts resembling schizophrenia[6]. SHULGIN[7] testing a number of TMA derivatives among them the present 3,4,5-analogue reported the drug was twice as active as mescaline. The present investigation therefore sought to determine acute behavioral, biochemical and adrenal (adrenocortical and adrenomedullary) influences of 3,4,5-trimethoxyamphetamine in male albino mice to observe a possible common pattern and relationships of hallucinogenic substances.

The compound 3,4,5-trimethoxyamphetamine HCl (TMA) was synthesized by the method of HEY[8]. To observe acute effects of TMA on behavior and endocrine activity, male albino mice (CFW) averaging 25 g were matched by body weights into appropriate test and control groups after prior acclimatization for 1 week in cages containing 4 mice per cage. Test animals were injected i.p. with TMA solutions at dose levels of 50 mg/kg (Group A) and 100 mg/kg (Group B). Control mice received equivalent injections of saline.

Effects on locomotor activity were evaluated in open-field enclosures[4] for 0–40 min at 5 min intervals in aliquot groups of mice and in additional groups from 40 to 150 min after administration of the single injections of TMA and/or saline solutions. Aliquot groups of test and control mice were likewise sacrificed by rapid decapitation 40 min and $2^1/_2$ h after TMA administration. Heparinized blood specimens were collected and assayed for plasma glucose[9] and corticosterone[10] titers. The adrenals were excised

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