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EFFECT OF DIURETICS ON ENERGY METABOLISM

AKIRA YOSHIDA, TAKEO YAMADA* and SHOZO KOSHIKAWA
2nd Department of Medicine, Tokyo Medical and Dental University, Tokyo, Japan

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Abstract—The effects of diuretics (amiloride-HCl, hydrochlorothiazide, acetazoleamide, ethacrynic acid and chlormerodrin) on the electron transfer system and ATP formation system have been investigated with rat kidney. Chlormerodrin and ethacrynic acid not only suppressed O_2 uptake of renal slices and renal cortical mitochondria, but also inhibited the respiration which had been accelerated by 2,4-DNP with renal cortical mitochondria. Furthermore, the observed P:O ratio was slightly decreased by both the diuretics. These results suggest that chlormerodrin and ethacrynic acid mainly have an inhibitory action upon the electron transfer system and an effect of reducing the efficiency of ATP formation in part.

THE Na^+ REABSORPTION of renal tubule is by active transport which requires the expenditure of metabolic energy.[1] Although the diuretics to suppress the active transport have been described, the precise mechanism is not fully elucidated. Many studies were reported concerning the effects of diuretics on the ATP utilization and ATP formation.[2–11] Most of the studies paid much attention to the inhibition of ATP utilization by diuretics,[2–8] while a few reported on the effects on the electron transfer and ATP formation systems.[9–11] The present study was undertaken to investigate the effects of diuretics on both the electron transfer and ATP formation systems on the following bases: (1) studies of various effects of diuretics on the O_2 uptake in the renal slices; (2) studies of effects of diuretics on the O_2 uptake and P:O ratio in renal cortical mitochondria prepared from nontreated rats; (3) studies of O_2 uptake and P:O ratio in renal cortical mitochondria prepared from rats pretreated with diuretics; (4) studies of effects of chlormerodrin and ethacrynic acid on the rate of O_2 uptake accelerated by 2,4-DNP in renal cortical mitochondria.

MATERIALS AND METHODS

Preparation of slices from rat kidney cortex and medulla. Male Wistar rats, 150–200 g were decapitated after 24 hr starvation. The kidneys were excised and after removal of the capsule, each was bisected longitudinally and the cortex and medulla separated and sliced with a sharp knife. All preparation was performed at 4°.

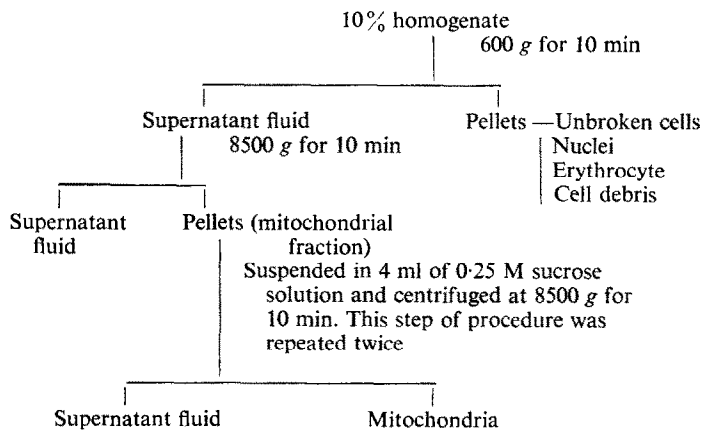
Preparation of kidney mitochondria. Mitochondria were prepared by modified Weinbach's method.[12] Two g of renal cortex were mixed with 18 ml of 0·25 M sucrose solution and the mixture was homogenized for 1 min in a teflon homogenizer at 1000 rev/min. The resultant 10% homogenate was fractionated by centrifugation at 2°. The schematic representation of all procedures is shown in Table 1.

* Present address: Department of Medicine, Jōsai Dental College, Saitama Japan.

Measurement of the rate of O_2 uptake. Oxygen uptake was measured polarographically at 20° with Yanagimoto's oxygen electrode in 2 ml cell. For study with slices, a special sealed cell is devised to adapt to the oxygen electrode.

Calculation of P:O ratio. The P:O ratio and the respiratory control index were calculated from the polarographic tracings of respiration by the method of Chance and Williams.[13] P:O ratio is equivalent to micromoles ADP added/microatoms oxygen utilized.

TABLE 1



Reaction medium. In the case of slice experiments, the reaction medium contained 0·21 M mannitol, 10 mM KCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$ and 10 mM Na-phosphate buffer (pH 7·4). In the case of mitochondrial experiments, the reaction medium contained 10 mM tris (pH 7·4) and 5 mM Na-phosphate (pH 7·4) buffer, 50 mM KCl, 8 mM $MgCl_2$ and mannitol. The osmotic pressure of the solution was adjusted by the mannitol to 250 m-osmoles/l.

Substrate. Succinate was used as substrate in slice experiments and both succinate and α-ketoglutarate were used as substrate in the studies of the effects of diuretics on the O_2 uptake and P:O ratio in renal cortical mitochondria.

Kinds and doses of the diuretics added or administered. The diuretics used were amiloride-HCl (referred to hereafter as MK-870), hydrochlorothiazide (HCT), acetazoleamide (AA), ethacrynic acid (EA) and chlormerodrin (Hg). In the case of experiments on addition of diuretics, the concentration of all diuretics added was 3×10^{-4} M in slice, and between 5×10^{-5} and 5×10^{-4} M in mitochondrial experiments.

In experiments of administration of diuretics, daily doses were as follows: MK-870 4·0 mg/kg, HCT 10 mg/kg, AA 100 mg/kg, EA 15 mg/kg and Hg 4·0 mg/kg respectively. These diuretics were administered intraperitoneally for 14 days. For compensation of potassium depletion, about 50 ml/day of 2 mM KCl solution were administered orally, except in the case of MK-870.

The concentration of 2,4-DNP added was 1×10^{-5} M. Protein concentration of mitochondria was determined by the method of Folin,[14] using bovine serum albumin as the standard.

RESULTS

Effects of diuretics on the O_2 uptake in the renal slices

Figure 1 shows the effects of diuretics on the O_2 uptake of the slices of renal cortex and medulla. The heavy line represents the rate of O_2 uptake of cortical slices, and the broken lines that of medullar slices. The effects of diuretics were similar with both the medulla and cortex. Neither MK-870 nor AA exhibited any significant influence upon the O_2 uptake, whereas EA and Hg evidently exhibited inhibitory effects. The effect of HCT was slightly inhibitory.

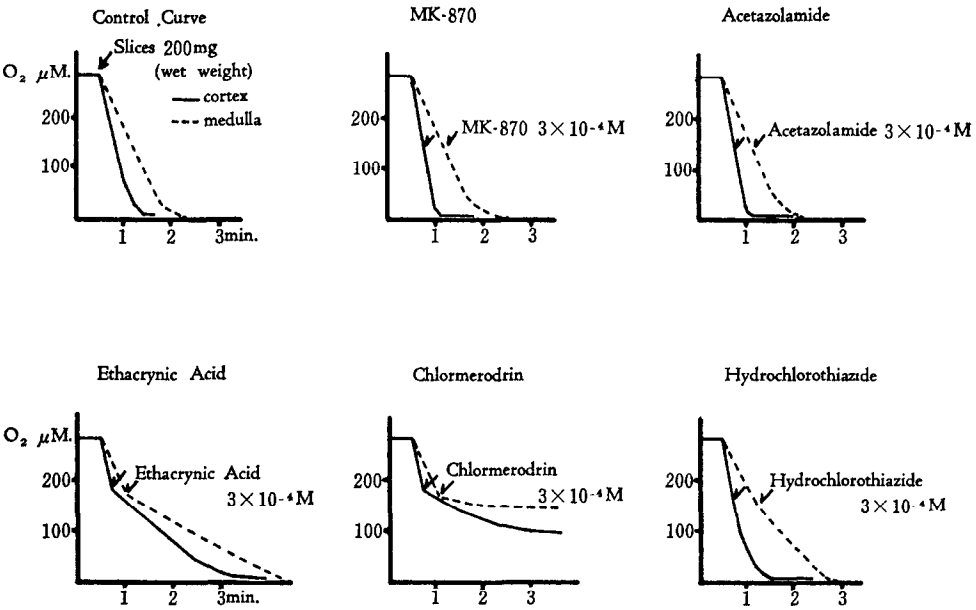


FIG. 1. Effect of diuretics on oxygen consumption of renal slices (*in vitro*).

Effects of diuretics on the O_2 uptake and P:O ratio in untreated mitochondria

Figure 2 shows the polarographical tracing of O_2 uptake of the renal cortical mitochondria prepared from untreated rats, when succinate was used as substrate. Addition of ADP stimulated the respiratory rate in the oxidation of succinate. The cycles of stimulated respiration in the presence of ADP and decreased respiration following the exhaustion of ADP could be repeated several times. Figure 3 shows the tracing when α-ketoglutarate was used as substrate. In this case, the rate of O_2 consumption at both state 3* and state 4† was smaller than that when succinate was used as substrate. From these polarographic tracings and the amounts of ADP added, the rate of respiration and P:O ratio were calculated, as described above. Table 2 presents these values obtained in the presence and absence of diuretics. The left half of Table 2 presents the effects of diuretics when succinate was used as substrate and the right

* State 3; ADP-stimulated respiration, during ATP formation.
† State 4; respiration after ADP utilized to ATP formation.

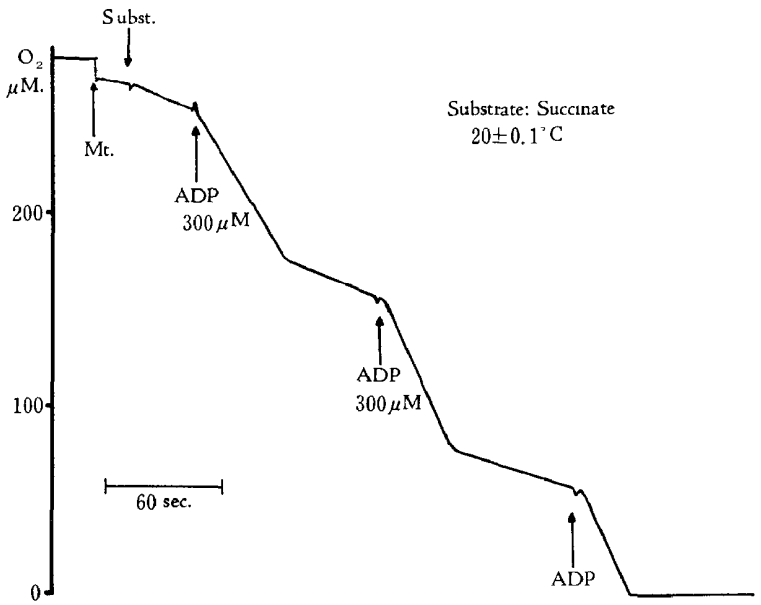


FIG. 2. Respiration of renal mitochondria.

half the values obtained using α-ketoglutarate as substrate. From this table, it seems likely that EA suppresses not only the respiration at state 3 and state 4, but decreases the P:O ratio. When Hg was added in the concentration of 1×10^{-5} M, the suppression of O_2 uptake and lowering of P:O ratio were apparent. When the concentration of Hg was raised to 1×10^{-4} M, the suppression of the rate of O_2 uptake was

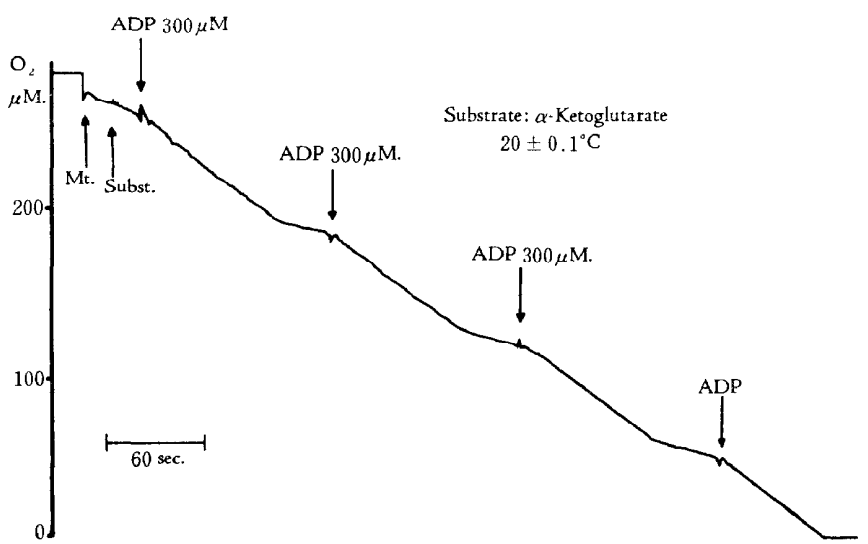


FIG. 3. Respiration of renal mitochondria.

TABLE 2. EFFECT OF DIURETICS ON OXIDATIVE PHOSPHORYLATION OF RENAL MITOCHONDRIA

Added diuretics		Succinate			α-Ketoglutarate		
		O_2 consumption			O_2 consumption		
		State 3	State 4	P:O	State 3	State 4	P:O
None	—	111·0 ± 10·0*	27·2 ± 4·2	1·62 ± 0·03	58·6 ± 6·0	15·4 ± 2·0	2·30 ± 0·10
MK-870	10^{-5}M	113·6 ± 9·0	26·0 ± 4·0	1·63 ± 0·05	58·2 ± 5·0	14·8 ± 1·8	2·31 ± 0·09
	10^{-4}M	108·8 ± 8·0	20·6 ± 4·2	1·69 ± 0·04	58·0 ± 4·0	14·8 ± 2·2	2·30 ± 0·09
Hydrochlorothiazide	10^{-5}M	107·2 ± 10·0	23·6 ± 2·6	1·65 ± 0·10	57·4 ± 4·0	14·2 ± 1·8	2·34 ± 0·06
	10^{-4}M	110·4 ± 14·0	20·0 ± 4·6	1·64 ± 0·09	56·0 ± 4·8	14·4 ± 2·0	2·32 ± 0·08
Acetazoleamide	10^{-5}M	104·6 ± 2·0	27·0 ± 1·0	1·65 ± 0·10	58·0 ± 4·0	14·8 ± 2·0	2·30 ± 0·10
	10^{-4}M	101·0 ± 2·2	25·2 ± 1·0	1·58 ± 0·03	59·0 ± 4·4	15·2 ± 1·2	2·31 ± 0·08
Ethacrynic acid	10^{-5}M	97·2 ± 6·0	24·8 ± 3·0	1·55 ± 0·05	50·6 ± 4·0	12·8 ± 1·8	2·20 ± 0·04
	10^{-4}M	90·4 ± 10·0	24·0 ± 2·0	1·52 ± 0·05	46·4 ± 4·8	12·2 ± 2·4	2·15 ± 0·07
Chlormerodrin	10^{-5}M	85·4 ± 15·4	22·4 ± 2·0	1·39 ± 0·05	44·0 ± 3·6	11·6 ± 1·6	1·97 ± 0·10

* Results are given ± S.E. O_2 consumption (mμM/mg/min).

so marked that the calculation of P:O ratio was impossible. The effects of the diuretics when using α-ketoglutarate as substrate are quite similar to those with succinate. Hg significantly and EA slightly brought about suppression of the rate of O_2 uptake and lowering P:O ratio. These effects, however, were not observed by using the other diuretics such as MK-870, HCT and AA.

Studies of O_2 uptake and P:O ratio in renal cortical mitochondria prepared from rats pretreated with diuretics

Table 2 summarizes the results obtained from experiments of administration of diuretics. Both EA and Hg suppressed the rate of O_2 uptake at both state 3 and state 4 and lowered the P:O ratio slightly. These effects were not, however, observed with other diuretic agents. The results obtained using α-ketoglutarate as substrate are similar to those obtained using succinate as substrate.

Effects of Hg and EA on the rate of O_2 uptake accelerated by 2,4-DNP in renal cortical mitochondria

The experimental results described above suggest that EA and Hg suppress the respiration of renal slices and mitochondria with reduction in efficiency of ATP formation. Therefore, the mode of this inhibitory action was subsequently studied.

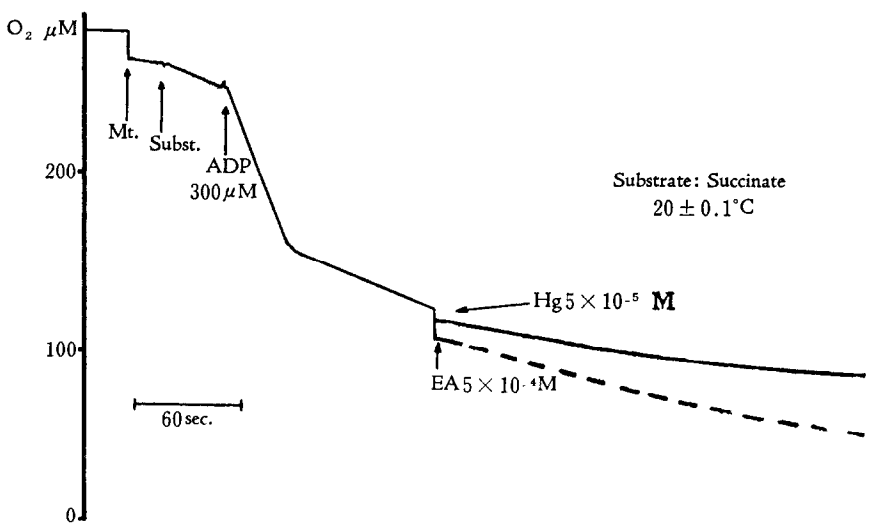


FIG. 4. Effect of chlormerodrin and ethacrynic acid on respiration of renal mitochondria.

Figure 4 shows the respiratory states 3 min after the addition of 5×10^{-4} M of EA and 5×10^{-5} M Hg, respectively, to mitochondria at state 4. Both EA and Hg remarkably suppressed the respiration of mitochondria at state 4. Figure 5 shows the effects of EA and Hg on the action of 2,4-DNP which is known to accelerate respiration of state 4 by uncoupling high energy intermediates. As can be seen in the figure, the respiration accelerated by 2,4-DNP was significantly suppressed by Hg at the concentration of 5×10^{-5} M Hg and 5×10^{-4} M EA respectively.

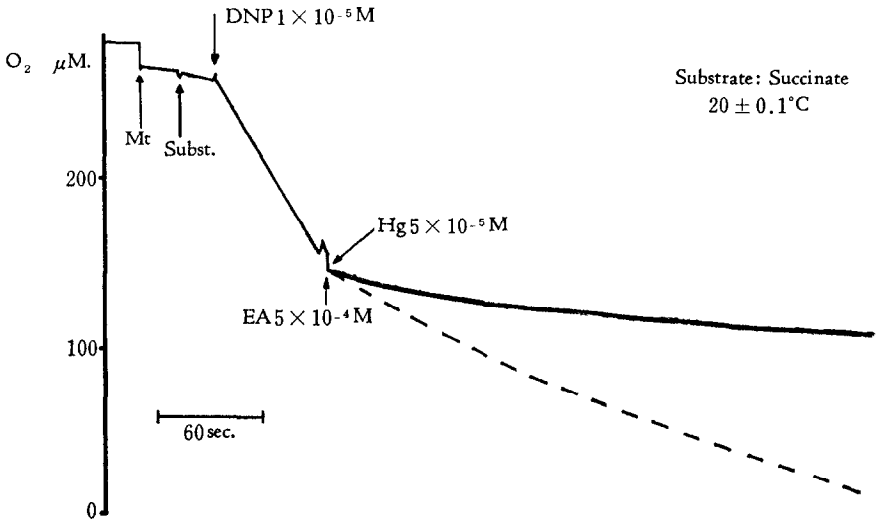


FIG. 5. Effect of chlormerodrin and ethacrynic acid on respiration of renal mitochondria.

DISCUSSION

The Na^+ reabsorption of renal tubule is by active transport which is carried out by energy utilization. The energy source responsible for active ion transport is not fully elucidated. Two hypotheses can be proposed, depending on whether the energy is supplied by ATP or not, that is, the ATPase hypothesis[2–8,15] or the other hypothesis which demands an alternative pathway of transformation of chemical energy to transport work.[11,20] According to the ATPase hypothesis, the energy liberated from the breakdown of ATP by membrane ATPase is utilized for Na^+ reabsorption. The other hypothesis seeks the source of energy in energy liberated from the electron transport system.

It is a well known fact, however, that the oxygen consumption of the kidney is high. This is generally explained to be attributable mainly to Na^+ reabsorption. The relation between the oxygen consumption and transport of electrolytes was initially observed by Krammer and Deetjen who demonstrated the correlation between GFR and oxygen consumption.[16] Later it was shown by many investigators[17–20] that there exists a linear relationship between the Na^+ reabsorption and the O_2 consumption. The existence of a linear relationship between Na^+ reabsorption and O_2 consumption suggests that the greater part of energy necessary for Na^+ transport is derived from aerobic process.

Moreover, it is generally accepted that respiration and ATP formation take place mainly in the granules (mitochondria) within cells.[21] Morphologically, most of the mitochondria are arranged along the basement membrane of renal tubular cells, especially in the position where the active transport of Na^+ takes place, i.e. proximal tubules, ascending limbs of the Henle's loops and distal tubules.[22] These findings indicate that mitochondria have a very important role in the active transport of Na^+ in the renal tubules. Namely, if the electron transfer system or ATP formation system is directly inhibited, the active transport of Na^+ would be depressed.

TABLE 3. EFFECT OF DIURETICS ON OXIDATIVE PHOSPHORYLATION OF RENAL MITOCHONDRIA

Added diuretics	Injected dose (mg/kg/day)	Succinate			α-Ketoglutarate			n
		O_2 consumption			O_2 consumption			
		State 3	State 4	P:O	State 3	State 4	P:O	
None	—	111·0 ± 10·0*	27·2 ± 4·2	1·62 ± 0·03	58·6 ± 6·0	15·4 ± 2·0	2·30 ± 0·10	14
MK-870	4	98·8 ± 7·0	26·0 ± 3·0	1·60 ± 0·06	58·8 ± 5·2	15·0 ± 1·0	2·40 ± 0·11	6
Hydrochlorothiazide	10	97·9 ± 7·6	25·2 ± 3·8	1·60 ± 0·09	58·0 ± 9·2	14·7 ± 3·0	2·30 ± 0·10	6
Acetazoleamide	100	106·4 ± 6·0	26·0 ± 4·0	1·67 ± 0·05	55·6 ± 2·0	14·2 ± 2·0	2·35 ± 0·08	6
Ethacrynic acid	15	93·6 ± 4·0	24·6 ± 3·0	1·52 ± 0·05	45·2 ± 8·0	13·6 ± 3·0	2·10 ± 0·11	6
Chlormerodrin	4	83·6 ± 2·0	13·8 ± 2·0	1·45 ± 0·04	38·4 ± 2·0	9·6 ± 2·0	1·97 ± 0·05	6

* Results are given ± S.E.

O_2 consumption (mμM/mg/min).

Jones and Landon investigated the effects of meralluride and ethacrynic acid on glycolysis and respiration of rat kidney slices.[8] These authors suggested that mitochondrial respiration could be the primary site of action of ethacrynic acid, based on the finding that EA inhibited respiration at lower levels than that required to inhibit glycolysis, but they did not investigate the effects of the diuretics on respiration in mitochondria itself. We studied the effects of many kinds of diuretics on the oxidative phosphorylation in mitochondria in addition to slice experiments and studied further the mode of the action of the diuretic agents that inhibit the rate of O_2 uptake in slice and in mitochondria.

Among five diuretics only EA and Hg not only suppressed the rate of O_2 uptake in the slice experiments, but suppressed the rate of O_2 uptake in mitochondrial experiments (Fig. 1, Tables 2 and 3). In the experiments of O_2 uptake in renal cortical mitochondria prepared from rats pretreated with these diuretics, both EA and Hg also revealed an inhibitory effect. From these findings it must be assumed that both EA and Hg have an inhibitory effect on intracellular respiration. Moreover as shown in Fig. 5, both these diuretics (EA and Hg) suppressed the rate of mitochondrial respiration accelerated by 2,4-DNP. This result suggests that these diuretics have an inhibitory action on the electron transfer system.

On the other hand, there is a possibility that these diuretics (Hg and EA) seem to have an inhibitory effect on ATP formation system, since Hg and EA slightly lowered P:O ratio in renal mitochondria from prepared rats both pretreated and untreated by diuretic. Greif and Jacobs[10] reported that chlormerodrin lowered the P:O ratio in renal mitochondria prepared from normal kidney, but in the case of administration of ^{203}Hg labelled-chlormerodrin 4 hr prior to killing, chlormerodrin failed to lower the P:O ratio. In our experiments, diuretics involving chlormerodrin were administered for 14 days prior to killing. Chlormerodrin suppressed the rate of O_2 uptake and P:O ratio in renal mitochondria pretreated by this diuretic. Duration and dosage of agents administered are different from each other. These differences might be mentioned to explain the contrast in results.

SUMMARY

Both chlormerodrin and ethacrynic acid not only suppressed the rate of O_2 uptake of rat renal slices and cortical mitochondria, but inhibited the rate of O_2 uptake which had been accelerated by 2,4-DNP with rat renal cortical mitochondria.

Both chlormerodrin and ethacrynic acid slightly lowered the P:O ratio in renal mitochondria prepared from normal rats.

A slightly lower P:O ratio was observed in mitochondria prepared from rats pretreated by chlormerodrin and ethacrynic acid respectively.

These results seem to suggest that chlormerodrin and ethacrynic acid have an inhibitory action mainly upon the electron transfer system and an effect of reducing the efficiency of ATP formation in part.

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