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EFFECTS OF INDOMETHACIN ON THE METABOLISM OF GLUCOSE BY ISOLATED RAT KIDNEY TUBULES

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The demonstration by Whitehouse (1) that indomethacin, a non-steroidal anti-inflammatory drug, uncoupled oxidative phosphorylation in isolated rat liver mitochondria has been amply confirmed (2 - 4) and the suggestion has been made that membrane sulphydryl groups are involved in this effect (2). In addition indomethacin can induce mitochondrial swelling through increasing mitochondrial permeability to alkali metal cations (3) and a recent report states that the drug also inhibits the electron transport chain at a site above cytochrome c (4).

Such actions of indomethacin hint at the possibility that it might influence cell metabolism in ways which are unrelated to its role as an inhibitor of prostaglandin synthesis (5). A previous report by one of us (6) showed that cylate, another anti-inflammatory drug, affected m- at kidney tubules by uncoupling oxidative est, therefore, to investigate whether ıbule metabolism and, if so, whether ts uncoupling action.

ules and their use in metabolic previously (6 - 8). Tubules (9mg phosphate-buffered medium (6) on vessels shaken continuously ıd 2,4-dinitrophenol (2,4-DNP) ıse of its low solubility in ı ethanolic solution to give systems containing ethanol few experiments were also

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performed in which indomethacin was introduced in aqueous solution. Oxygen consumption was measured manometrically (9). Enzymatic methods were used for the determination of lactate (10), pyruvate (11) and glucose (12). Glucose measurements were not affected by indomethacin even though it has been reported that indomethacin inhibits peroxidase, one of the enzymes involved in the assay procedure (13). Possibly most of the indomethacin was removed during acid deproteinization of the incubation mixtures. $^{14}CO_2$ evolved during the metabolism of D-[U-^{14}C] glucose, [1-^{14}C]pyruvate or L-[1-^{14}C]lactate was trapped in 2N-NaOH and determined by liquid scintillation spectrometry after precipitation as $Ba^{14}CO_3$ on glass fibre discs (8). Protein was measured by the method of Lowry et al.(14).

The effects of 2,4-DNP and indomethacin on the consumption of oxygen by isolated kidney tubules provided with D-[^{14}C]glucose as the sole exogenous substrate are shown in Fig.1. 2,4-DNP slightly increased

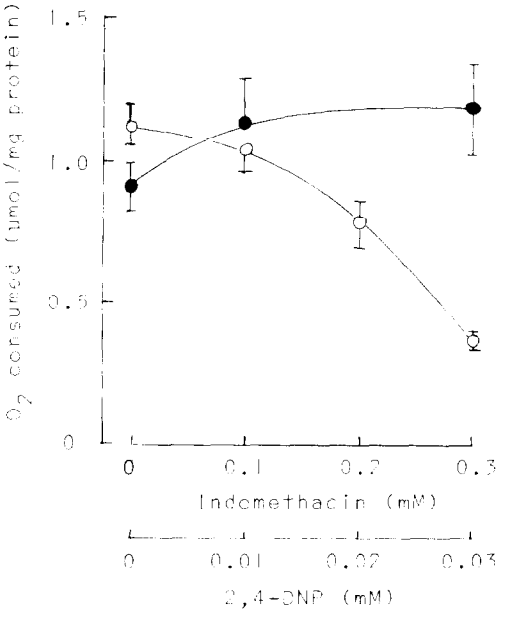

Fig.1. Effects of indomethacin and 2,4-DNP on oxygen consumption by tubules metabolizing glucose. Tubules were incubated for 1h at 37° in 2ml of buffered medium containi 0.1 µCi of D-[U-^{14}C]glucose (1mM) and either indomethac or 2,4-DNP (●) as indicated. Each point represent value ± S.E.M. for 6 - 8 separate experiments.

the rate of oxygen uptake, an effect which was

uncoupling activity. In contrast, indome+

previously found to uncouple oxidative phosphorylation in isolated mitochondria (1 - 4), inhibited oxygen consumption. This strong inhibition did not seem to be caused by direct inhibitory effects on either the tricarboxylic acid cycle or the mitochondrial respiratory chain since respiration with 5mM 2-oxoglutarate as the added substrate was virtually unaffected by indomethacin though 2,4-DNP again stimulated oxygen consumption by about 40% (data not shown).

The above results showed that indomethacin inhibited the ability of glucose to support respiration without directly affecting oxidative mechanisms in the mitochondria. In order to determine how this effect was exerted a study was made of the influence of indomethacin, and of 2,4-DNP, on glucose metabolism; the results are presented in Fig. 2.

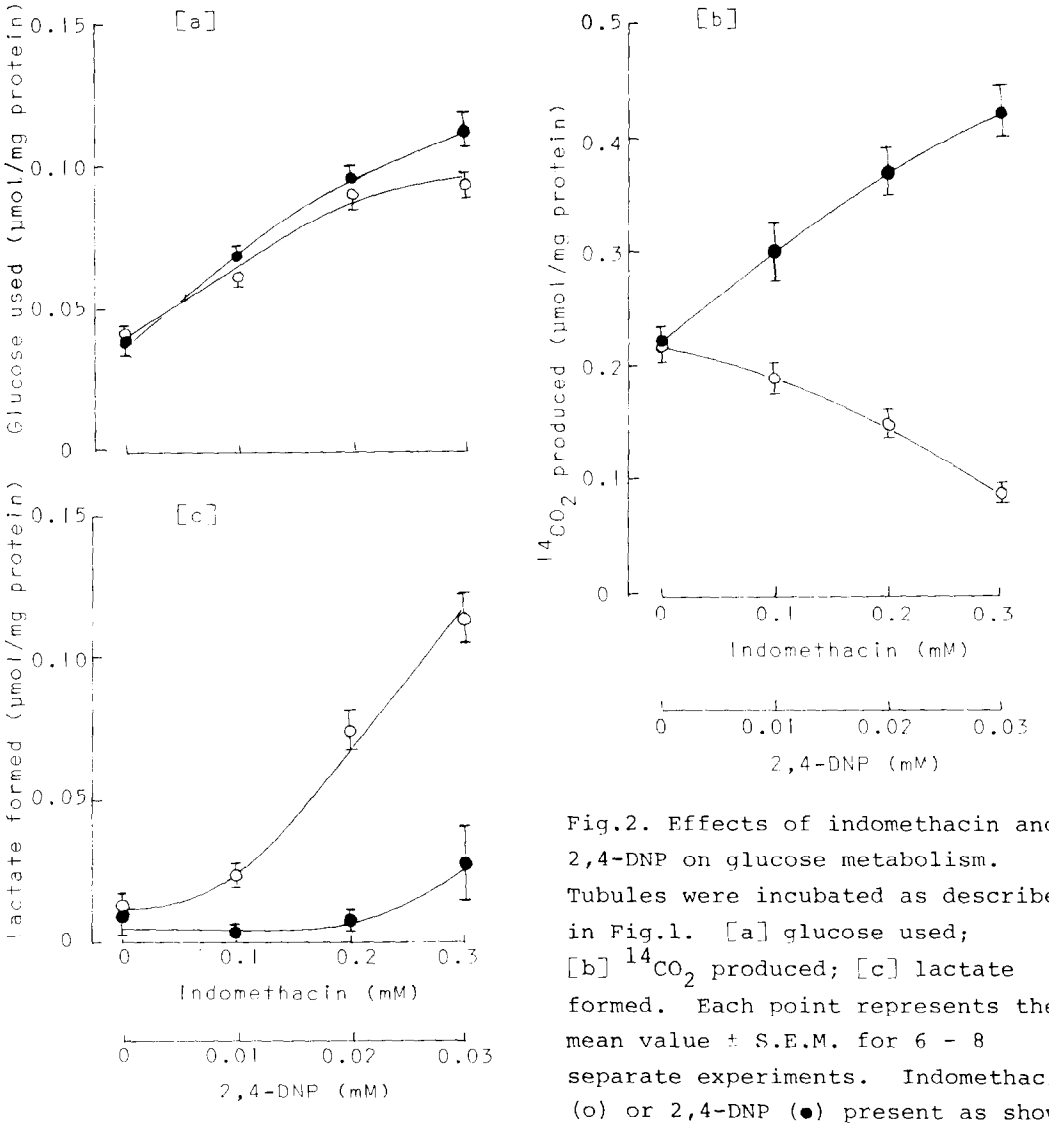

Fig.2. Effects of indomethacin and 2,4-DNP on glucose metabolism. Tubules were incubated as described in Fig.1. [a] glucose used; [b] $^{14}CO_2$ produced; [c] lactate formed. Each point represents the mean value ± S.E.M. for 6 - 8 separate experiments. Indomethacin (o) or 2,4-DNP (●) present as shown.

Both indomethacin and 2,4-DNP stimulated the use of [^{14}C]glucose by kidney tubules (Fig. 2a). With 2,4-DNP the increase in [^{14}C]glucose metabolism was accompanied by an increase in the liberation of $^{14}CO_2$ while the amount of lactate formed was small. In contrast, indomethacin inhibited the oxidation of [^{14}C]glucose to $^{14}CO_2$ but strongly stimulated its conversion to lactate (Fig. 2b,c). These results indicated that, whereas 2,4-DNP allowed the oxidation of glycolytically-produced pyruvate, indomethacin promoted its reduction to lactate. Further experiments revealed that this effect of indomethacin was not exerted on the metabolism of exogenously supplied pyruvate. Neither the oxidation of added [1-^{14}C]pyruvate to $^{14}CO_2$ nor its conversion to lactate was significantly altered by indomethacin though a slight increase in oxygen consumption occurred when indomethacin was present (Fig. 3). From this it was

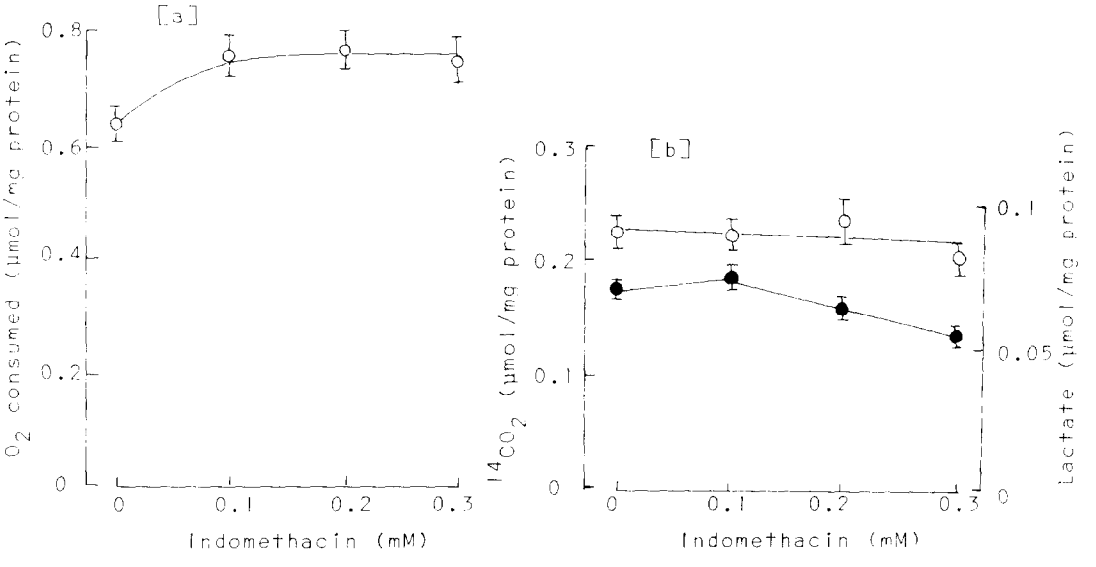

Fig.3. Effect of indomethacin on pyruvate metabolism. Tubules were incubated for 30 min at 37° in 2ml of buffered medium containing 0.1 µCi of [1-^{14}C]pyruvate (2.5mM). [a] oxygen consumed; [b] $^{14}CO_2$ produced (o) and lactate formed (●). Each point represents the mean value ± S.E.M. for 8 separate experiments.

concluded that although indomethacin inhibited the oxidation of pyruvate produced from glucose, it did not do so by interfering directly with the mechanisms involved in pyruvate oxidation. Instead the effect of indomethacin had to be looked upon as a positive stimulation of pyruvate reduction which lowered the amount available for oxidation.

It may be speculated that the diversion of glycolytically-produced pyruvate to lactate is the consequence of an increase in the cytoplasmic $NADH/NAD^+$ ratio when indomethacin is present since the equilibrium position of the lactate dehydrogenase reaction is determined by this ratio (15). An increase in the $NADH/NAD^+$ ratio can not be attributed to any increase in ethanol oxidation since indomethacin was found to affect glucose metabolism in the same way whether it was added in aqueous or ethanolic solution. Moreover, alcohol dehydrogenase activity in the rat kidney cortex is low and ethanol appears not to influence carbohydrate metabolism in this tissue (16). An attractive hypothesis to account for an increase in the $NADH/NAD^+$ ratio is that indomethacin inhibits the transfer of cytoplasmic reducing equivalents, produced during glycolysis, to the mitochondria via the glycerol phosphate and/or malate "shuttles" (17). Results consistent with this idea are given in Fig. 4. Here it can be seen that the metabolism of L-[1-^{14}C]lactate

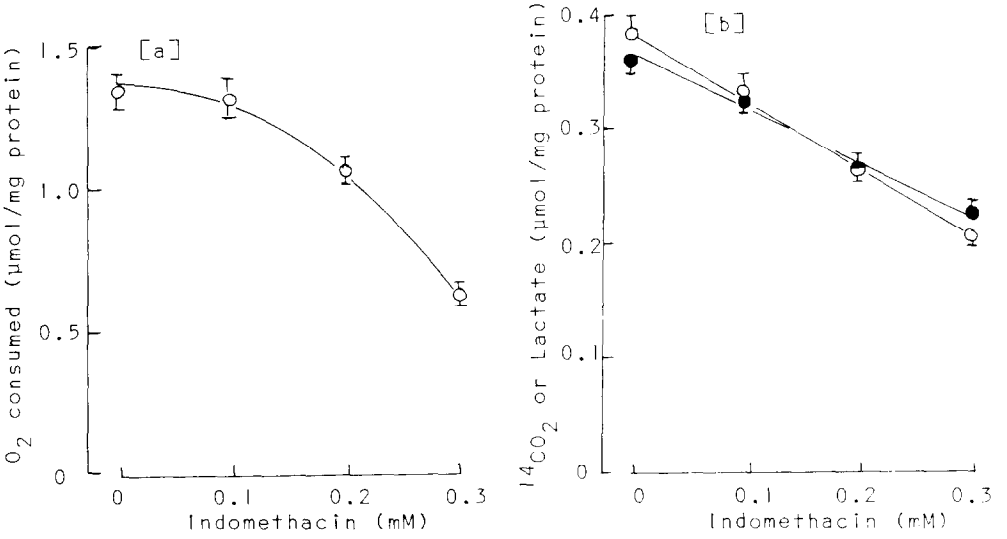

Fig. 4. Effect of indomethacin on lactate metabolism. Tubules were incubated for 1h at 37° in 2ml of buffered medium containing 0.1 µCi of L-[1-^{14}C]lactate (2.5mM). [a] oxygen consumed; [b] $^{14}CO_2$ produced (o) and lactate consumed (•). Each point represents the mean value ± S.F.M. for 6 separate experiments.

was affected by indomethacin in the same way as was the metabolism of glucose. Oxygen consumption by tubules metabolizing lactate was inhibited by indomethacin (Fig. 2a) as were the overall consumption of lactate and its conversion to $^{14}CO_2$ (Fig. 2b). Since it was shown earlier that pyruvate oxidation was unaffected by indomethacin it

may be concluded that the inhibited step in lactate oxidation is its conversion to pyruvate. This is consistent with the hypothesis that indomethacin interferes with the oxidative disposal of cytoplasmically-produced reducing equivalents.

In the light of the above results it seems worthwhile to pursue investigations into the possibility that indomethacin inhibits the transfer of reducing equivalents from cytoplasm to mitochondria and such investigations are currently under way in this laboratory. It is clear that despite the acknowledged uncoupler activity of indomethacin, its effects on glucose metabolism in kidney tubules contrast markedly with those of the classical uncoupler 2,4-dinitrophenol and can not, therefore, be explained simply in terms of an uncoupling action.

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