could not be shown between E^{1}_{cp} values and respiratory inhibition. Other chemical and/or physicochemical parameters must be involved in the inhibitory process. In particular the lipophilic character of the molecules might be important in determining the penetration through the mitochondrial membrane. At the present time we are carrying on further experiments with submitochondrial particles in order to study the possible relationship between lipophilicity and inhibitory effects of the compounds.

In summary, 2.3 micromoles/mg protein of MFNI induced a 60% decrease in the heart mitochondrial ADP-stimulated oxygen uptake using glutamate–malate as substrate. The same amount of niridazole, ipronidazole, DA 3851 and ornidazole led to falls of less than 20% in the oxygen uptake, whilst metronidazole was ineffective. State 3 and state 3 u (uncoupled) respiration were affected to the same extent. Oxygen-uptake using succinate as substrate was not inhibited indicating that the action was exerted at the NADH oxidation level. The relationship between electroreduction potentials of the test compounds and inhibition of respiration has been studied.

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The effect of various calmodulin inhibitors on the response of adrenal glomerulosa cells to angiotensin II and cyclic AMP

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Involvement of calcium ions in the stimulatory action of angiotensin II, corticotropin (ACTH) and potassium ions on adrenal glomerulosa cells has been amply verified [1]. Nevertheless, the precise role of calcium ions in the stimulation of adrenal steroidogenesis is still a matter of controversy. In several tissues many calcium-mediated cell activities have been shown to be controlled by the calcium-dependent regulator protein calmodulin [2]. In a previous study [3] we demonstrated that the tranquillizer drug trifluoperazine, an inhibitor of calmodulin, completely prevented the aldosterone-stimulating action of angiotensin II and potassium ions, both agents supposedly acting via calcium ions rather than cyclic AMP (cAMP). At the same concentration trifluoperazine inhibited only partially the response to ACTH or its second messenger cAMP. These results raise the possibility that calmodulin may participate in the stimulation of aldosterone production. Moreover, it was also assumed that calmodulin plays a different role in the mode of action of angiotensin II and potassium ions on the one hand and of ACTH or cAMP on the other hand. To test whether the effect of trifluoperazine was due to the inhibition of calmodulin in the present experiments we examined the effect of three calmodulin antagonists of partly heterologous structure (penfluridol, pimozide and trifluoperazine) chosen on the basis of the studies of Weiss *et al.* [4]. In order to compare the mode of action of stimuli

acting either via cAMP or a calcium signal, the effect of these drugs was examined on cells stimulated with dibutyryl cAMP (db-cAMP) and angiotensin II, respectively.

Materials and methods. Materials used for cell isolation as well as for aldosterone analysis have been described [5]. Angiotensin II (Asp^1-Val^5-angiotensin II-β-amide, Hypertensin) was obtained from Ciba-Geigy (Basle, Switzerland), db-cAMP from Calbiochem (Lucerne, Switzerland), penfluridol and pimozide from Jansen Pharmaceutica (Beerse, Belgium) and trifluoperazine from Smith Kline & French Laboratories (Philadelphia, PA).

Glomerulosa cell suspensions were prepared from the adrenal capsular tissue of male Sprague–Dawley (CFY) rats after treatment with collagenase, as detailed by Enyedi and Spät [5]. The yield was 200,000 glomerulosa cells per rat on average (N = 7).

The cells (about 100,000 per sample) were incubated in Teflon vials at 37° for 90 min in an atmosphere of 95% O_2 and 5% CO_2. The incubation was carried out in 1-ml mixtures of Medium 199 (Wellcome, Beckenham, U.K.) and Krebs–Ringer bicarbonate glucose solution (1:2, v/v) containing 2 g human serum albumin [fraction V (Humán, Budapest, Hungary)] per litre. Potassium concentration was 3.6 mM. Penfluridol and pimozide were dissolved in dimethylsulfoxide; the final concentration of the latter was 0.5% and control samples contained the same concentration of the solvent. Each incubation was carried out in duplicate.

Aldosterone content of the cell suspension was estimated by radioimmunoassay as previously described [5].

The effect of various drugs on the basal production rate of aldosterone was expressed as a per cent of the control. Inhibition of the response to different stimuli by these antagonists was expressed as a per cent of the maximal response:

$$\% \text{ of the maximal response} = 100 \times \frac{\text{stimulated in the presence of inhibitor} - \text{basal}}{\text{stimulated in the absence of inhibitor} - \text{basal}}$$

Means ± S.E.M. are given. The lines were drawn by eye.

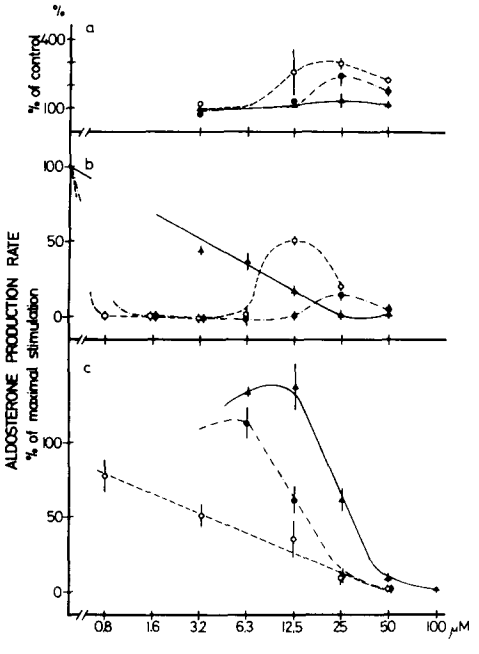

Fig. 1. Effect of penfluridol (- - - ○ - - -), pimozide (- · · · -●- · · · -) and trifluoperazine (——▲——) on the production rate of aldosterone by isolated rat glomerulosa cells. (a) Non-stimulated cells (N = two duplicates), (b) cells stimulated with 25 nM angiotensin II (N = three duplicates) and (c) cells stimulated with 100 μM dibutyryl cAMP (N = three duplicates). Production rate of aldosterone is expressed as per cent of the control in panel a and as per cent of the maximal stimulation evoked by the agonists in panels b and c. Means and range in panel a and means ± S.E.M. in panels b and c are shown.

Results and discussion. In seven separate experiments the basal production rate of aldosterone was 5.4 ± 0.7 pmoles/10^5 cells. All three tranquillizer drugs increased the production rate but not to the same extent. Penfluridol was more efficient than pimozide, while trifluoperazine evoked only a very slight (about 30%) stimulation (Fig. 1a).

Angiotensin II at a concentration of 25 nM caused a 5.6 ± 0.25-fold increase in the production rate; this response was strongly antagonized by the three drugs. The concentration of trifluoperazine which reduced the response by 50% (IC_{50}) was about 3–4 μM. Penfluridol and pimozide applied at 0.8 and 1.6 μM concentrations, respectively, totally blocked the stimulatory action of angiotensin II. Higher concentrations of the two drugs increased again the production rate as they did in non-stimulated cells (Fig. 1b). The effects of lower concentrations of penfluridol and pimozide (0.05–3.2 μM) were examined in two further experiments. The stimulation of aldosterone production in response to 25 nM angiotensin II (4.6- and 6-fold) was completely blocked by 0.8 μM of either drug, while the IC_{50} value was about 0.2 μM in the case of both antagonists (data not shown).

The 12.4 ± 2.3-fold stimulation evoked by 100 μM db-cAMP was slightly enhanced by low concentrations of trifluoperazine and pimozide but not by penfluridol. Applied at higher concentrations, however, all three drugs inhibited this stimulation (Fig. 1c). Penfluridol, pimozide and trifluoperazine reduced the response to db-cAMP by 50% at respective concentrations of 5, 15 and 30 μM. These concentrations are considerably higher than the IC_{50} values estimated in cells stimulated with angiotensin II.

Inhibition of aldosterone production by these calmodulin antagonists is compatible with our previous assumption [3] that calmodulin plays a role in the control of glomerulosa cell function. However, tranquillizer drugs may exert their effects independently of calmodulin. In addition to the inhibition of calmodulin-dependent enzyme activity [4] they were found to inhibit phospholipid-sensitive Ca-dependent protein kinases in various tissues [6]. Comparison of the inhibitory action of these drugs on calmodulin- and phospholipid-sensitive Ca-dependent enzymes showed that the respective IC_{50} values were considerably higher in the case of the latter enzymes and the relative order of potency of antagonists was different for the two groups of enzymes [6]. In the present experiments IC_{50} values were as low or even lower than those estimated by Weiss *et al.* [4] for calmodulin-dependent brain phosphodiesterase. The relative order of potency of the drugs was similar in both systems. As to the well-known dopamine receptor antagonistic effect of the drugs used in our study, stimulation of glomerulosa cells by angiotensin II, potassium ions or ACTH was found not to be influenced by metoclopramide [7].

Stimulation of aldosterone production was observed for a well-defined concentration range of each antagonist. This observation renders it possible that besides stimulatory control mechanisms inhibitory control mechanisms of aldosterone production are also affected by calmodulin; however, non-specific effects cannot be ruled out.

There are only a few data in the literature concerning the role of calmodulin in the adrenal cortex. Harper *et al.* [8] were the first to demonstrate the presence of calmodulin in this tissue by an immunofluorescent technique. Hall *et al.* [9] found in murine adrenal tumor cells that calmodulin

was involved in the steroid-stimulating action of ACTH and its second messenger cAMP, influencing mitochondrial cholesterol uptake and cholesterol side-chain cleavage enzyme activity. Calmodulin-induced phosphorylation of certain acceptors in adrenocortical mitochondrial fragments was observed by Bristow *et al.* [10].

All these data together suggest that calmodulin plays a role in the control of adrenocortical steroidogenesis. Since the stimulation with db-cAMP was less sensitive to the inhibitory action of these drugs than that of angiotensin II, it may be presumed that the mechanism of calmodulin action differs in the case of stimuli acting via calcium signal from those activating the cAMP system.

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Time and dose dependence of 3-methylcholanthrene-induced metabolism in rat intestinal mucosal cells and microsomes

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Oxidative drug metabolism activity in the intestinal mucosa is considerably less than in the liver [1, 2]. It is, however, easily inducible by a variety of compounds present in cigarette smoke and certain foods [3–5]. After induction intestinal monooxygenase activity becomes of quantitative importance as was recently shown by Klippert *et al.* [6] in our department. They demonstrated an intestinal first-pass deethylation of phenacetin in rats after induction with 3-methylcholanthrene (3-MC)*.

In order to estimate the *in vivo* activity of intestinal monooxygenase, several systems including everted sacs [7, 8], isolated mucosal cells [9–11], homogenates [3] and microsomal fractions [12, 13] thereof have been used.

Activity observed in these systems may differ due to variations in the availability of cofactors and diffusion barriers for substrates or their metabolities. Moreover, the inducers and their metabolites may accumulate in cells and/or may interfere with cell wall integrity or biochemical processes which are needed for maintenance of monooxygenase activity.

We studied the time course of induction by 3-MC and Arochlor 1254. It appears that considerable differences occur in both the magnitude and time course of the induction between cells and microsomes, which can be explained by toxic effects of the inducing agent 3-MC in cells.

* Abbreviations: 3-MC, 3-methylcholanthrene; 7-EC, 7-ethoxycoumarin; 7-HC, 7-hydroxycoumarin; LDH, lactate dehydrogenase.

Materials and methods. Adult male Wistar rats weighing approximately 250 g (TNO, Zeist, The Netherlands) were used. Pretreated animals received a single intragastric injection of 1.0 ml corn-oil or 3-MC, 5–20 mg/kg body weight, in 1.0 ml corn-oil by stomach tube, 24 or 48 hr before preparation of cells and/or microsomes. Arochlor 1254, 50–200 mg/kg, was administered in the same manner. After treatment rats were allowed free access to food and drinking water.

Preparation of intestinal mucosal cells was basically the same as described by Hülsmann *et al.* [14, 15]. The gut was divided into four 15-cm lengths, everted on metal rods, attached with surgical silk and exposed to longitudinal vibration (100 Hz, 2-mm amplitude) using a Vibro-Mixer (Chemap AG, Mannedorf, Switzerland). Isolated cells were suspended in ice-cold Krebs–Ringer medium saturated with carbogen gas, pH 7.4. Microsomes were prepared from isolated mucosal cells as described by Shirkey *et al.* [16] using an Ultra-Turrax (Janke & Kunkel KG, Staufeni, Breisgau) as the homogenizing apparatus. Cell viability was measured by LDH leakage from the cell cytoplasm into the medium and at least 2 hr after isolation was still between 80 and 90% [10].

The *O*-dealkylation of 7-EC was determined according to Greenlee and Poland [17]. Conjugated 7-HC was deter-