RNA-synthesis in rat kidneys after fluorinated anesthetics or inorganic fluoride administration[1]

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Summary. RNA-synthesis in rat kidneys is affected by the administration of 0.5% methoxyflurane and 1% enflurane; also fluoride, a product of fluorinated anesthetics metabolism, hinders renal RNA-synthesis in rats.

Kidney and liver lesions after fluorinated anesthetics administration have well been documented by clinical and experimental studies[2].

An experimental model[3,4] has been proposed in rats in which a fluorinated anesthetic, methoxyflurane, caused a dose-dependent kidney lesion and the injection of inorganic fluoride produced renal functional and morphological abnormalities similar to those caused by methoxyflurane anesthesia.

Studies on the effect of fluorinated anesthetics on RNA-metabolism in vivo are scarce[5]: the present study was undertaken to investigate the effect on RNA-synthesis in rat kidneys after methoxyflurane anesthesia in conditions leading to renal damage[4], and its effects were compared with those caused by halothane and enflurane or by inorganic fluoride.

Experimental. Male Wistar rats weighing 160–180 g were used. Water and food were allowed ad libitum and room temperature was maintained at 22–24 °C. For anesthetic treatment, animals were placed in a closed plastic chamber[3]; 0,5% methoxyflurane (Abbott S.P.A., Campoverde, Latina Italy), 1% halothane (Icpharma, Milano, Italy) and 1% enflurane (Abbott) were vaporized for 180 min with Pentec Mk II, Fluotec Mk III and Enfluratec vaporizers, respectively, employing oxygen and nitrous oxide (33 and 66% v/v) as carrier gas at a flow rate of 4 l/min. Control rats received carrier gas (N_2O and O_2) at the same flow and for the same time. Treated and untreated animals were left for 1 h to open air before killing.

Inorganic fluoride was administered by s.c. injection of a 0.2 M NaF solution (1 ml) in distilled water to normal unanesthetized rats[4]. Control rats received the same volume of distilled water.

RNA-synthesis was measured by the incorporation of 6-[^{14}C]-orotic acid (sp. act. 57 mCi/mmole, Amersham, Buchs, England) into RNA. Orotic acid was given by i.p. injection at the dosage of 3 µCi/100 g of b.wt. Rats were killed 20 min later. RNA was extracted and measured according to Munro and Fleck[6].

Results and discussion. The effect of anesthesia with different fluorinated compounds on kidneys RNA-synthesis is reported in figure 1.

In the method of Munro and Fleck[6], the radioactivity in the supernatant after the first acid precipitation includes the labelled precursor and its metabolites that entered the cell but were not yet incorporated into RNA: the radioactivity of this fraction is therefore considered an index of the cellular uptake[7]. The unchanged radioactivity in this fraction (figure 1) suggests that the cellular uptake is unaffected after the administration of anaesthetics.

On the contrary, the incorporation into RNA of the labelled compound varies with the anesthetic used: methoxyflurane causes a 50% inhibition of RNA-synthesis; the 16% inhibition present in the group of rats treated with enflurane becomes statistically significant ($p < 0.02$) when the data are corrected for the acid-soluble fraction. Corrected values are calculated dividing the specific activity (dpm/mg RNA) by the acid soluble radioactivity (dpm/mg tissue).

Halothane also decreases the incorporation into RNA of the labelled precursor but the 20% inhibition is not significant, even after correction for the acid-soluble fraction. Fluoride is a metabolite of methoxyflurane and enflurane in the rat and man[8–10] and mimics some of methoxyflurane effects in rats[4].

2 h after fluoride administration (figure 2) RNA-synthesis is reduced to 17% of the control value; this inhibition is progressively reduced to 36% at 4 h, but also the cellular uptake of the orotic acid is modified. Corrected values are therefore presented (figure 2, third column of each group) to better evaluate the phenomenon: from these data RNA-synthesis is reduced to 42% and to 63% of the control at 2 and 3 h, respectively, but at 4 h the synthesis returns to normal values.

The data presented show that fluorinated anesthetics interfere with RNA-synthesis in rat kidneys: the highest inhibition of RNA-synthesis is obtained after treatment with

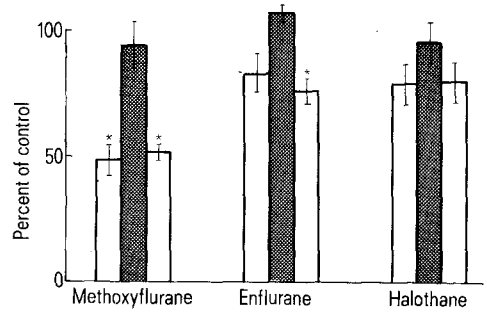

Fig. 1. Effect of fluorinated anesthetics on RNA-synthesis in rat kidneys. Results are expressed as percent of control values. First column: RNA-synthesis; hatched column: acid-soluble radioactivity; third column: RNA-synthesis, corrected values. Control values were 8503±720 dpm/mg RNA for RNA-synthesis; 865±76 dpm/mg tissue for acid soluble radioactivity and 10.15±0.95 for corrected values. Results are expressed as means±SEM of at least 5 animals for each group. Statistically significant differences (Fisher's $p < 0.05$) are indicated by an asterisk. Corrected values have been obtained dividing the specific activity (dpm/mg RNA) by acid-soluble radioactivity (dpm/mg tissue) measured in the supernatant after the first acid precipitation in the method of Munro and Fleck[6].

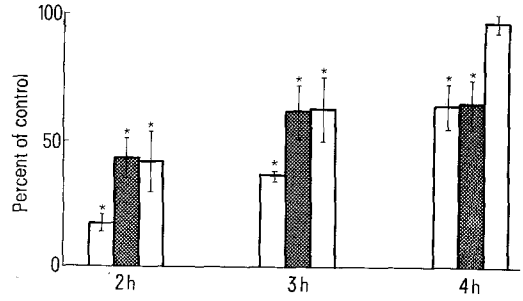

Fig. 2. Effect of NaF-administration on RNA-synthesis in rat kidneys. Percentages of control values are expressed as means±SEM of at least 4 animals. Rats were killed 2, 3 and 4 h after fluoride or distilled water injection; because no difference in RNA-synthesis among control values was detectable, results were pooled. All other indications are as in figure 1.

methoxyflurane 0.5%, enflurane gives only 16% inhibition and halothane effects are scarce and not statistically significant. The different inhibition of RNA-synthesis can be ascribed to higher blood levels of inorganic fluoride after methoxyflurane than after enflurane or halothane treatment in rats[8-10].

The data reported in figure 2 indicate that sodium fluoride parallels to a certain extent the methoxyflurane effect causing an early inhibition of RNA-synthesis, but the pattern of this inhibition differs in some features from that caused by the anesthetic: methoxyflurane does not alter the orotic acid uptake into kidney cells and the inhibition by fluoride, that is marked at 2 and 3 h, is no longer evident at 4 h after NaF administration on the basis of corrected values.

The data presented in this paper suggest that in kidneys an early event after methoxyflurane or NaF administration is the inhibition of RNA-synthesis, which may be responsable of the degenerative changes described in kidney proximal tubules after methoxyflurane or fluoride treatment[4]. The inhibited synthesis of RNA may be a result of other derangements in cellular metabolism, although no alteration in subcellular structures of the kidney cells was detected[4] immediately after methoxyflurane anesthesia.

1 Acknowledgments. This work was supported by a grant from C.N.R., Roma.
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Mechanical responses of the isolated cervix of the day-22 pregnant rat to field stimulation[1]

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Summary. Field stimulation of isolated, spirally-cut cervix from day-22 pregnant rats produced contractions which could be inhibited by tetrodotoxin or hyoscine and potentiated by propranolol. The rat cervix would appear to receive both cholinergic and noradrenergic innervations whose transmitters activate muscarinic cholinoceptors and β-adrenoceptors respectively.

Transmural stimulation of isolated uterine horns of non-pregnant rats demonstrated cholinergic contractions, noradrenergic inhibitions and possibly the presence of at least a 3rd neurotransmitter[2]. Cholinergic contractions of isolated, luminally perfused cervix of non-pregnant rats have also been produced by transmural stimulation[3]; inhibitory responses to transmural stimulation were not studied.

Histochemical studies also suggest cholinergic and noradrenergic myometrial and cervical innervations in the rat[3,4] whose density decreases during pregnancy[4,5]. Near term the density of noradrenergic innervation of the guinea-pig cervix[6] and the cholinergic innervation of the rat cervix[5] appear greater than those of the respective myometria. In the present study, field stimulation and antagonist drugs have been used to detect the presence and the likely nature of the neurotransmitters of any cervical innervation in the late pregnant rat.

Materials and methods. Spirally-cut cervical preparations from Sprague-Dawley rats on day 22 of gestation (day of finding copulation plug = day 1) were set up in modified Krebs at 37 °C as described previously[7]. A Grass S8 stimulator was used to apply field stimulation to the cervix via a pair of parallel stainless steel ring electrodes, 1 cm apart, with pulses of 0.5 msec and of supra-maximal voltage (60 V). After obtaining constant contractions to an acetylcholine maximum, stimuli were applied in ascending frequencies from 1 to 64 Hz using 10-sec trains every 100 sec. Tissues were incubated in solutions containing modifying drugs for 30 min before agonist drugs and stimulation were repeated. The Wilcoxon matched pair signed rank test[8] was used to test the significance of differences.

Results and discussion. Field stimulation produced single phasic contractions of the cervix with initial responses seen to 4 or 8 Hz, depending on the tissue, and amplitude increasing with frequency. The maximal response of the cervix to field stimulation was 68.8±5.6% (mean±SEM; n = 37) of that of an acetylcholine maximum.

Tetrodotoxin (3.1×10^{-7} moles/l) clearly antagonized the contractions to field stimulation at low-pulse width (figure) suggesting that the responses were nerve-mediated. Field stimulation of spirally-cut uterine horns from the same animals did not consistently produce contractions and these were not significantly antagonized by tetrodotoxin. This could be due to the much greater spontaneous contractility of the uterine horns or their lesser density of innervation compared to the cervix. Hyoscine (1×10^{-8} moles/l) antagonized responses to field stimulation suggesting that the cervix possesses a cholinergic innervation whose transmitter acts on muscarinic cholinoceptors. Responses at 32 and 64 Hz were potentiated by propranolol (1×10^{-8} moles/l) but not modified further by addition of phentolamine (1×10^{-7} moles/l) to the propranolol. This indirectly suggests some noradrenergic innervation whose transmitter acts solely on β-adrenoceptors to produce relaxation.

The antagonistic properties of the modifying drugs were tested by measuring their effects on the responses to various agonists. Hyoscine was selective in that it produced an 83-fold antagonism of acetylcholine contractions but left