Results and discussion. The serum AFP level of ethionine-treated rats increased sharply after 2 days lag. The increases in serum AFP level and liver triglyceride content in female rats were identical with those of male rats when tested at the age of 35 days. However, the extent of the increases in adult rats were more prominent in the female rats. Although no significant elevation of serum GPT activity was found during the entire course of ethionine treatment, rises of liver triglyceride content and G6PD activity indicate the existence of liver cell injury[3]. The incorporation of ^{3}H-thymidine into liver DNA was rather diminished up to 2 days, followed by a gradual and small increase at 3 to 4 days after the treatment (Figure). Administration of ATP to ethionine-treated male as well as female rats effectively inhibited the rise of not only serum AFP concentration but also liver triglyceride content and G6PD activity. These observations suggest that the elevation of serum AFP in ethionine-treated rats is not directly related to the stimulated synthesis of hepatic DNA.

A comparatively lower dose of thioacetamide has been reported to produce increased hepatic DNA synthesis and liver cell proliferation without any detectable cell damage[4]. Changes of these biochemical parameters for liver cell injury in rats treated with a lower dose of thioacetamide were also found to be minimal. The increase in serum AFP concentration was insignificant, even after a marked stimulation of liver DNA synthesis which reached a maximum level in 2 days after the treatment. The results indicate that the low dose of thioacetamide caused neither any evidence of liver cell injury nor increased production of AFP, even in the presence of accelerated DNA synthesis in liver.

Our recent studies have shown that the increase in serum AFP level following partial hepatectomy is relatively small as compared with that after CCl_4 treatment, and that the underlying mechanisms are also different[2]. Accordingly, the derepression of AFP genome associated with liver cell injury itself appears to play a major role in the increased AFP production in injured liver. This hypothesis is in accord with the results of our previous studies of key carbohydrate-metabolizing enzymes in damaged livers in the sense that the extent of enzyme deviation in injured livers is closely similar to that in undifferentiated hepatocytes[5].

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Increase in Membrane Conductance by Adrenaline in Parotid Acinar Cells

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Summary. It is shown that excitation of the α- or β-adrenoceptors in mouse parotid acinar cells causes a marked reduction of surface cell membrane resistance. The α-adrenoceptor induced membrane effect is an increase in K conductance. The β-adrenoceptor induced membrane effect does not seem to be mediated by cyclic AMP.

Using intracellular micro-electrode recording from parotid acini it has recently been shown that there are three distinct receptors influencing the acinar cell membrane potential. Excitation of a cholinergic (muscarinic) receptor or an α-adrenoceptor causes hyperpolarization whereas excitation of a β-adrenoceptor results in depolarization[1]. The mechanism of action of acetylcholine on salivary acinar cells has been thoroughly investigated and there is little doubt that ACh acts by increasing the cell membrane conductance to K^+ and possibly also to Na^+[2,3]. With respect to the mechanism of action of

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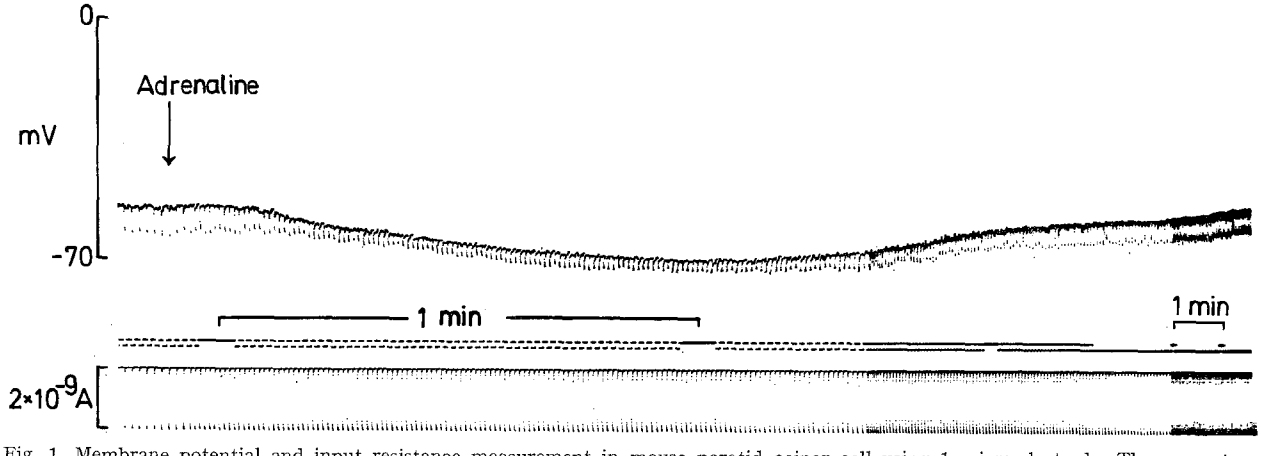

Fig. 1. Membrane potential and input resistance measurement in mouse parotid acinar cell using 1 micro-electrode. The upper trace shows the spontaneous membrane potential and the short-lasting hyperpolarizations caused by the rectangular current pulses (100 msec duration), injected through the recording micro-electrode, shown in the lower trace. Adrenaline added to the tissue bath in a single dose to achieve for a short period a concentration of 10^{-5} *M* markedly hyperpolarized the cell membrane and reduced the amplitude of the short-lasting current-pulse-induced hyperpolarizations.

adrenaline on parotid acinar cells SELINGER et al.[4] concluded that the adrenaline-induced K^+ release was an energy requiring process.

PETERSEN and PEDERSEN[1] have suggested that the initial step in the α-adrenergic membrane action is to increase membrane K^+ permeability. In smooth muscle adrenaline seems to act by increasing the K^+ permeability[5].

I have now for the first time examined the effect of adrenaline and isoprenaline on the parotid acinar cell membrane resistance and also examined the possibility that the isoprenaline effect could be mediated by cyclic AMP since it is known that isoprenaline is a powerful stimulant of parotid adenyl cyclase[6].

Methods. The experiments were carried out on mouse parotid segments in a superfusion bath[1]. Membrane potentials and resistance between cell interior and the external medium were measured using 1 microelectrode through which current pulses could be injected as previously described in detail[7,8].

Results. Figure 1 shows the marked reduction in input resistance occurring concomitantly with the hyperpolarization following adrenaline stimulation. In the resting state the mean input resistance was 3.1 ± 0.2 $M\Omega$, this was reduced to 1.0 ± 0.2 $M\Omega$ ($n = 16$) ($p < 0.001$) by adrenaline (10^{-5} M). This marked reduction in input resistance was associated with a reduction in membrane time constant (Figure 2) from about 5–10 to 2–3 msec. It is therefore reasonable to conclude that the reduction in input resistance is due to a reduction of the surface cell membrane resistance and not to a change in the junctional cell membrane resistance[8].

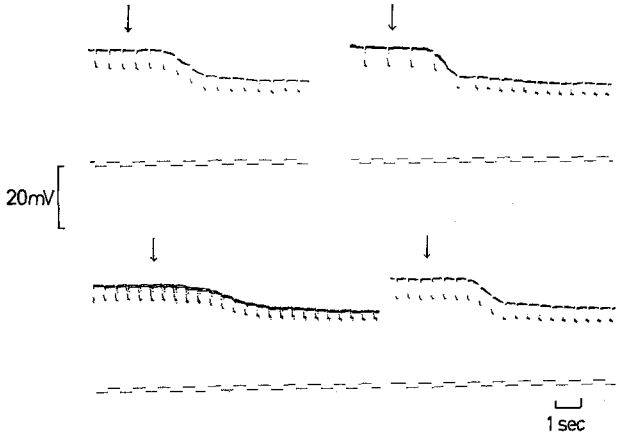

Fig. 2. Four examples of the effect of adrenaline (10^{-6} M) (↓) on membrane potential and resistance. Current pulses of constant size and duration (2×10^{-9} A, 100 msec) were used throughout (not shown). Note quickening of the time course of the current pulse induced membrane hyperpolarizations during the action of adrenaline.

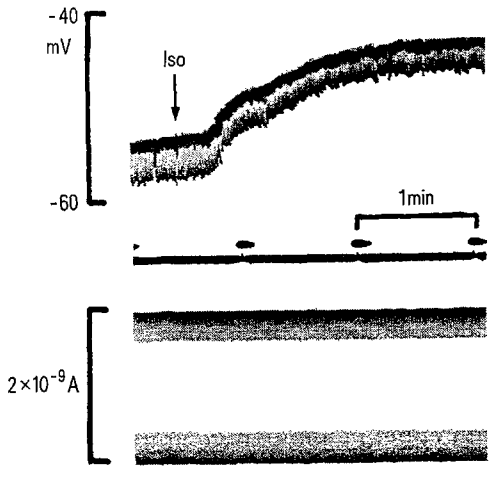

Fig. 3. Effect of isoprenaline (10^{-5} M) on cell membrane potential and input resistance.

The hyperpolarization induced by adrenaline is caused by α-receptor activation[1]. Activation of the β-receptor by isoprenaline, resulting in depolarization, was accompanied by a reduction in input resistance from 2.4 ± 0.3 to 1.2 ± 0.3 $M\Omega$ ($n = 6$) ($p < 0.05$) (Figure 3).

Since isoprenaline is a powerful stimulant of parotid adenyl cyclase it seemed possible that the membrane effect of isoprenaline was mediated by intracellular cyclic AMP as seems to be the case for the glucagon-induced liver cell membrane hyperpolarization[9]. However, the effect of exogenous dibutyryl cyclic AMP was different from that of isoprenaline. Dibutyryl cyclic AMP (2 mM) caused a mean hyperpolarization of 3.5 ± 1.1 mV ($n = 6$) while isoprenaline (10^{-5} M) in the same preparations caused a depolarization with a mean value of 9.4 ±1.1 mV ($n = 7$). No effect on the membrane potential was observed using lower concentrations of cyclic AMP (0.1 to 1 mM).

Discussion. The results obtained indicate that the membrane hyperpolarization evoked by α-adrenergic stimulation is due to an increase in membrane ion conductance. Since it has previously been shown that the amplitude of the hyperpolarization is very dependent on the extracellular K^+ concentration[1] it seems reasonable to conclude that α-adrenoceptor excitation in parotid acini causes an increase in cell membrane K^+ permeability. This agrees with earlier experimental results showing that adrenaline induces a marked K^+ release from perfused salivary glands[10].

Although it is known that isoprenaline activates an adenyl cyclase in the parotid[6] the results obtained may indicate that the isoprenaline-induced depolarization is not mediated by intracellular cyclic AMP accumulation, but it cannot be excluded that a high extracellular dibutyryl cyclic AMP concentration has other effects than intracellular cyclic AMP accumulation. Since exogenous cyclic AMP mimics the effects of isoprenaline on amylase secretion from the mouse parotid[11] the results at least indicate that the isoprenaline-induced potential and resistance change is not merely a consequence of the granule membrane insertion into the luminal plasma membrane occurring during the exocytosis process. The results obtained conform with the generalization that hormones and neurotransmitters evoke changes in cell membrane permeability and that this is an important step in their mechanism of action[12].

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