S-100 per g wet wt.)[15-16]. As the ratio was varied by changing the S-100 concentration in the tubes, an exponential gradient in the stimulating effect on the nucleolar RNA polymerase was observed. On the contrary, the nucleoplasmic RNA polymerase was not even stimulated by S-100, using such a concentration as 200 μg/ml which is twice as high as usually employed (Figure 2). Other proteins and synthetic polypeptides were also tested at the concentration of 100 μg/ml for their possible effect on RNA synthesis in isolated brain nuclei from 11-day chick embryos. Bovine serum albumin, ovalbumin, cytochrome C, poly-L-aspartate (M.W. 4,870), poly-L-glutamate (M.W. 19,700) had no stimulating effect. Different results were obtained by BONDY and ROBERTS with a different experimental system[17].

Further data on the selective stimulating effect of S-100 on the nucleolar RNA polymerase are presented in Figure 3. α-Amanitin, an inhibitor of the nucleoplasmic RNA polymerase, did not block the stimulating effect of the S-100 on the nucleolar RNA polymerase activity. Actinomycin D, that at low doses (0.1 μg/ml), primarily inhibits the nucleolar RNA polymerase, depresses proportionally both the control and the S-100-stimulated nucleolar RNA synthesis, suggesting that S-100 in some way antagonizes the inhibitory activity of this antibiotic. On the other hand, neither α-amanitin nor actinomycin D revealed any masked effect of the S-100 on the nucleoplasmic RNA polymerase. As might be expected, a relatively high concentration of actinomycin D (1 μg/ml) completely inhibited the nucleolar and the nucleoplasmic RNA polymerases, including the stimulating effect of S-100.

The above in vitro experiments on immature brain nuclei indicate that the S-100 protein stimulates the nucleolar RNA polymerase catalysing (pre-)rRNA synthesis, although a possible effect on the nucleoplasmic RNA polymerase, not detectable by the procedures used, cannot be completely excluded. This response in immature brain occurs at time when the endogenous S-100 is not yet accumulated in the developing chick brain[18-20]. The site(s) of action of the S-100 remains to be elucidated. Finally, we cannot yet attribute the stimulating effect of the S-100 to neuronal and/or glial nuclei since our preparations include both of them[21].

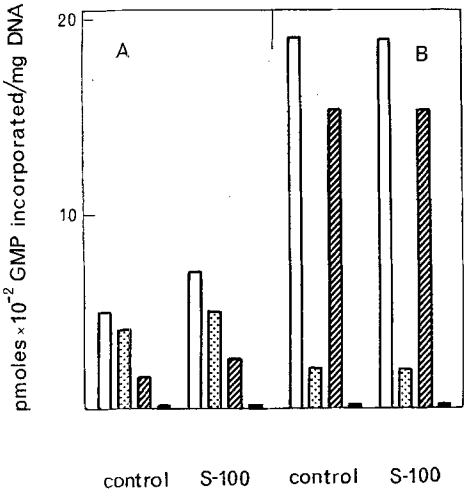

Fig. 3. In vitro effect of α-amanitin and actinomycin D on RNA polymerase activities stimulated by S-100 protein. The activity of the nucleolar (A) and nucleoplasmic (B) RNA polymerase was determined in isolated brain nuclei from 11-day chick embryos as described in the Table. S-100, when present, was at 100 μg/ml. α-Amanitin or actinomycin D, when present, was at the same concentration both in the preincubation and reaction mixtures. ☐ no antibiotic; ⁘ α-amanitin (2 μg/ml); /// actinomycin D (0.1 μg/ml); ■ actinomycin D (1 μg/ml). The set of experiments without antibiotics (□) is the mean value of 6 experiments. The other symbols are each the mean value of 3 experiments. Maximal variability per set of samples: ± 4.5%.

Riassunto. Una proteina specifica del sistema nervoso, chiamata S-100, stimola la RNA polimerase nucleolare in nuclei isolati da encefalo immaturo di pollo (11 giorni di incubazione). La proteina non ha alcun effetto sulla RNA polimerase nucleoplasmica del medesimo sistema sperimentale. La stimolazione della S-100 sulla RNA polimerase nucleolare è α-amanitina resistente ed è parzialmente antagonizzata dalla actinomicina. Ulteriori ricerche potranno chiarire il ruolo della proteina S-100 sulla espressione genetica del sistema nervoso.

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Intercellular Pathways in the Heart: Direct Evidence for Low Resistance Channels

Although indirect evidence[1] supports the view that impulse propagation in the heart is mediated by local circuit currents flowing through low resistance intercellular channels, other evidence[2] appears to contradict this hypothesis. In this report, by demonstrating the passage of ionized fluorescein dye from cell to cell, we present direct evidence for the existence of such intercellular pathways, and support for the former view.

Materials and methods. Rabbits and rats were prepared by a sharp blow on the neck or by ether anesthesia, respectively. Hearts were excised rapidly and the following tissues dissected: Interatrial septum and right atrial appendage of the rabbit; left and right atrial appendage and papillary muscle of the rat. The tissue was placed in a chamber mounted on the stage of a Leitz Ortholux microscope which was perfused with a bath containing the following ionic constituents in m*M* Na^+ 145; K^+ 4.2; Ca^{2+} 2.5; Cl^- 125.5; SO_4^{2-} 1.2; HCO_3^- 27; and dextrose 5.6. The bath was equilibrated with 95%

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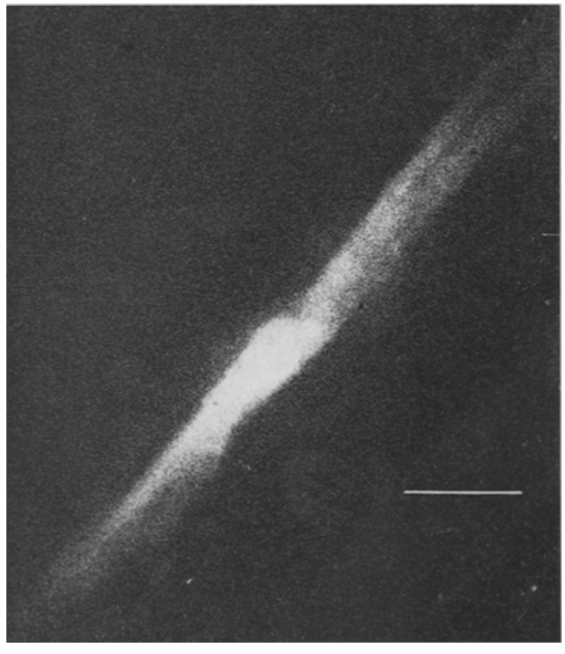
O_2-5% CO_2, circulated rapidly through the chamber, and maintained at 23 °C for the rat and 32 °C for the rabbit experiments.

Microelectrodes were filled with 0.1 *M* sodium fluorescein dye by boiling under low pressure; resistances were generally 20–30 Mohm. The electrodes were fixed coaxially to a cylindrical ultrasonic crystal which could be excited to vibrate axially[3]. Low amplitude vibration of the electrode (in the order of 50A) at 20–40 kHz caused the dye to be ejected from the microelectrode tip into the cell. A micromanipulator was used to advance the microelectrode-crystal combination until an intracellular potential was registered. The crystal was then driven for several sec until dye could be seen emerging from the tip using stereo microscopy with mercury vapor illumination. Both the resting potential and action potentials (in tissues which normally beat spontaneously) were monitored throughout the period of dye injection. The injection was considered successful if the resting potential decreased by less than 10 mV during dye infusion. When the cell appeared relatively bright, the electrode was withdrawn. The stage was then translated such that the cell could be observed with a Leitz Ultropak incident fluorescence illumination system with a 200 watt mercury vapor lamp, Leitz KP490 interference excitation filter, Leitz KP510 barrier filter, and an 11× Ultropak water immersion objective. Diffusion of dye was observed either through the binocular eyepiece or through a video system (COHU Model 2820 camera fitted with an SIT tube). Records were taken photomicrographically or with a video tape recorder.

The amount of fluorescein injected intracellularly could not be determined precisely[4]. However, fluorescein dye is brightest in concentrations of several hundred micromolar and can be detected in concentrations well below 0.1 μM. In our experiments, dye injection was terminated prior to the attainment of maximum brightness. Thus the maximum intracellular concentration, just after injection, was probably in the order of 100 μM or less.



Photomicrograph taken 1 min after microinjection of sodium fluorescein into a rat ventricular cell. The dye diffuses readily from cell to cell but does not diffuse into the extracellular space at detectable rates. Length reference 50 μm.

Results and discussion. A photomicrograph of a representative observation taken 1 min after injection into a rat ventricular cell is shown in the Figure. The dye is most concentrated in the cell into which it was injected, and is distinctly less concentrated in longitudinally contiguous areas. The concentration appears to fall off in a stepwise fashion, indicating specific diffusion barriers; presumably, by virtue of their spatial distribution, these are the intercalated discs which constitute the intercellular boundaries. Although the dye flows readily from cell to cell, it does not flow into the extracellular space at detectable rates.

After 5 to 15 min, depending upon the specific tissue, the dye diffused sufficiently that it could no longer be detected even in the primary cell. Results were consistent in all tissues studied, except in several rare cases (out of several hundred injections) in the interatrial septal preparation in which the dye did not diffuse appreciably out of the cell into which it was injected. In such cases, the dyed cell could be visualized easily for more than 60 min after injection with only a gradual loss of brightness.

The flow of fluorescein ions (MW 331, negatively charged) from cell to cell indicates that there are preferentially low resistance channels connecting adjacent mammalian myocardial cells. Although these channels have not been localized with certainty, probable sites are the hexagonal tubes which appear to connect contiguous cells at the nexal areas[5,6] of the intercalated discs.

Since fluorescein ions pass freely through intercellular channels, it is likely that ions of considerably lower molecular weight (e.g. potassium) will also pass. Indirect evidence for this has been obtained by WEIDMANN[7] who observed the diffusion of K^{42} along ventricular bundles. Presumably, the K^+ ions flowed through the intercalated discs. More recently, C^{14}-labelled tetraethylammonium bromide (TEA) has also been shown to diffuse along ventricular bundles[8].

Thus the low resistance intercellular connections needed for an impulse to propagate from cell to cell by local circuit (ionic) currents appear to exist. Furthermore, it seems likely that metabolites whose molecular weight is less than or equal to that of fluorescein may also pass readily from cell to cell.

Zusammenfassung. In einzelne Zellen von Kaninchen- und Rattenherzen wurde Natriumfluoreszin injiziert, wobei der Farbstoff direkt von Zelle zu Zelle diffundierte, aber nicht merklich auf den Extrazellulärraum übertritt. Daraus wird gefolgert, dass zwischen den Myocardzellen Verbindungswege existieren, von wo aus Impulse weitergeleitet werden können.

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