each case; in the single exception there was no significant difference in the response of the two sides. In 4 of the 8 cats, all of which showed a greater response in the denervated gland, the chorda lingual nerve to the normal gland was sectioned at the time of the acute experiment; in the other 4 it was left intact. Furthermore, in 2 cats the chronically denervated gland responded with vasodilatation during, as well as with additional vasodilatation after discontinuation of nerve stimulation. Except for the initial discovery of the sympathetic vasodilatation by CARLSON[7], it has never been reported during sympathetic nerve stimulation in the normal gland.

In 4 of the 8 experiments, in each of which the sympathetic nerve was stimulated at 10 Hz for 15 sec on both sides, the ratio of total blood flow between the denervated and control sides during the period of sympathetic after-dilatation ranged from 3.6–16.8 (mean, 8.6). Similar results were obtained with stimulation at 1–20 Hz for 5–20 sec.

The Figure shows the results of 2 separate experiments illustrating the typical increased sympathetic after dilatation in the chronically parasympathetically denervated gland. This enhanced vasodilatation, like that of the contralateral normal gland was unaffected by doses of atropine (500 µg/kg i.v.) or of propranolol (1–2 mg/kg i.v.) which blocked completely the hypotensive systemic actions of acetylcholine and isoprenaline, respectively.

The sympathetic vasoconstriction which occurs during the period of nerve stimulation, unlike the after-dilatation, was apparently unaffected by parasympathetic denervation. In general, it was related, both in the normal and denervated glands, to the basal blood flow, the total reduction being greater with the greater basal flows. There was also no evidence of its prolonged duration in the denervated gland (Figure).

The enhanced sympathetic after-dilatation following degenerative section of the pre-ganglionic parasympathetic nerve supply is an interesting observation. This effect, like the normal sympathetic after-dilatation, was also unaffected by BPF. Further, the kallikrein content of such chronically-denervated glands falls to approximately 5% of the normal gland[13]. These observations lead us to conclude that this enhanced sympathetic after-dilatation, like the normal one, is not mediated by kallikrein. Since both are unaffected by doses of atropine and propranolol which are effective in blocking the conventional muscarinic and β-adrenergic receptors[5], the increased response is not readily explicable in terms of the proliferation or increased responsiveness of such receptors. In our view, the mechanism of the normal sympathetic after-dilatation, and its enhancement which we have shown after preganglionic parasympathetic nerve section remain unexplained.

Zusammenfassung. Die «Nacherweiterung» der Gefässe, welche auf Reizung des sympathischen Nervs der Submaxillaris-Drüse in der Katze folgt, wird nicht durch Kallikrein oder Kinin ausgelöst, da sie nicht durch den hochwirksamen synthetischen Bradykinin-potenzierenden Faktors (BPF) beeinflusst wird. Eine neue Beobachtung zeigte, dass diese sympathische Gefässerweiterung bedeutend verstärkt wird, nachdem der parasympathische Nerv durchschnitten wurde und degeneriert ist.

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[13] S. BARTON, E. KARPINSKI and M. SCHACHTER, unpublished results.

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Effect of a Brain-Specific Protein (S-100 Protein) on the Nucleolar RNA Polymerase Activity in Isolated Brain Nuclei

The S-100 protein has been shown to be specific to the nervous system[1], and to conserve its immunological identity throughout phylogenesis[2–4]. The S-100 is a cytoplasmic component both of glia[5] and neurons[6], and in the neuroplasm it flows from soma to terminal. The function of the protein is unknown, but the possible involvement of the S-100 in neurophysiological functions[7–9] and behavioral parameters[10] has been suggested.

Data have been recently obtained on the presence of S-100 in brain chromatin and on the in vitro transfer of the protein into isolated nuclei[11]. In order to gain information about the role of the nuclear S-100, in the present report we describe the effect of the protein on the RNA synthesis in isolated nuclei from immature and mature brain. The results reported demonstrate that the S-100 stimulates the nucleolar RNA polymerase activity but not the nucleoplasmic RNA polymerase of immature chick brain.

Materials and methods. Nuclei from brain cortex of adult rabbit and immature brain of 11-day chick embryo were prepared as indicated elsewhere[11]. The activity of the nucleolar and nucleoplasmic RNA polymerases [Mg^{2+}-activated and Mn^{2+}-$(NH_4)_2SO_4$-activated enzymes] was determined as indicated in the Table[12]. The reaction of Mg^{2+}-activated and Mn^{2+}-$(NH_4)_2SO_4$-activated enzymes was arrested with 5 ml of ice-cold $HClO_4$ (0.5 N) in 1% of $Na_4P_2O_7$ or with 0.5 ml of ice-cold 10% CCl_3COOH plus 5 ml of ice-cold 5% CCl_3COOH in 1% of $Na_4P_2O_7$, respectively. 1 mg of bovine serum albumin was added as a carrier. The precipitate was washed twice more with 6 ml of cold 0.2 N $HClO_4$ in 1% of $Na_4P_2O_7$ or with 6 ml

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Effect of the S-100 protein on the nucleolar and nucleoplasmic RNA polymerase activities in isolated brain nuclei from adult rabbit and 11-day chick embryo

Nuclear type	Nucleolar RNA polymerase[a] (Mg^{2+}-activated enzyme)		Nucleoplasmic RNA polymerase[a] [Mn^{2+}-$(NH_4)_2SO_4$-activated enzyme]	
	Control	S-100	Control	S-100
Adult rabbit	480 ± 10 (4)	515 ± 10 (4)	1969 ± 58 (4)	2010 ± 65 (4)
Chick embryo	478 ± 16 (6)	660 ± 36 (6)	1808 ± 46 (8)	1794 ± 53 (8)

[a] *p*moles of GMP incorporated/15 min per mg DNA. The reaction mixture for the nucleolar RNA polymerase contained in a final volume of 0.5 ml: 0.1 *M Tris*-HCl buffer pH 8.0, 4 m*M* $MgCl_2$, 14 m*M* 2-mercaptoethanol, 0.6 m*M* each of ATP, CTP and UTP, 0.06 m*M* of ^{3}H-GTP (S.A. 100 Ci/mole). S-100 protein, when present, was at 100 µg/ml. The reaction mixture for the nucleoplasmic RNA polymerase contained in a final volume of 0.5 ml: 0.1 M *Tris*-HCl buffer pH 8.0, 4 m*M* $MnCl_2$, 0.28 *M* $(NH_4)_2$ SO_4, 0.6 m*M* each of ATP, CTP and UTP, 0.12 m*M* of ^{3}H-GTP (S.A. 50 Ci/mole). S-100 protein, when present, was at 100 µg/ml. For the assay of both RNA polymerases, aliquots of nuclear suspension were preincubated for 10 min at 37° in 0.1 *M Tris*-HCl, 4 m*M* $MgCl_2$, pH 8.0 in the presence or in the absence of S-100 protein (100 µg/ml). The assay was started in the reaction mixture with 0.1 ml of preincubated nuclei (~120 µg DNA) and the incubation was at 37 °C for 15 min. Mean results ± S.E.M. are given with the numbers of experiments in parenthesis.

cold 5% CCl_3COOH in 1% of $Na_4P_2O_7$, respectively. The final precipitate was solubilized in 1 ml of NCS reagent and 10 ml of toluene-based scintillation liquid were added for counting the radioactivity. ^{3}H-GTP (S.A. 5 Ci/m*M*) from New England Nuclear was tetrasodium salt. ATP, CTP, GTP and UTP (sodium salt) were purchased from Boehringer. Actinomycin D and α-amanitin were a gift of Merck, Sharp and Dohme and of Dr. DI MAURO, University of Rome. S-100 protein was prepared according to MOORE[1]. DNA was measured according to the method of BURTON[13].

Results and discussion. When isolated brain nuclei are incubated in the presence of S-100, the addition of divalent cations is required for the entry of the protein into the nuclei to proceed at a significant rate[11]. All the nuclear suspensions, therefore, were preincubated in the presence of $MgCl_2$ (4 m*M*) with or without the S-100 before the RNA polymerase activities were assayed. Under these conditions, in isolated brain nuclei of adult rabbit the nucleolar RNA polymerase was slightly stimulated by the S-100 protein (at limit of significance, $0.05 > P > 0.025$), but not the nucleoplasmic one (Table). These data are consistent with those obtained by DUTTON and MAHLER on rat brain[14]. On the other hand, in isolated nuclei from immature chick brain the nucleolar RNA polymerase (Mg^{2+}-activated enzyme) was markedly stimulated (~ 38%) by S-100, but not the nucleoplasmic RNA polymerase [Mn^{2+}-$(NH_4)_2SO_4$-activated enzyme]. These results on immature nuclei were confirmed by the kinetics of the 2 RNA polymerase activities in the presence or in the absence of S-100 (Figure 1).

In our reaction tubes the DNA to S-100 ratio (240 µg DNA/100 µg S-100 per ml) was comparable to that which occurs in brain tissue of adult rat (243 µg DNA/120 µg

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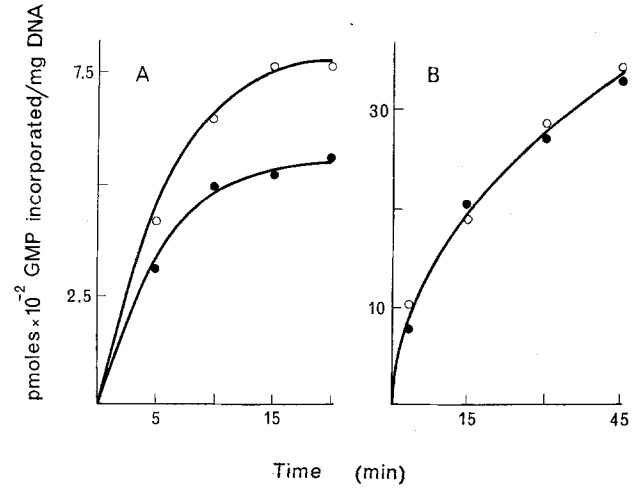

Fig. 1. Time-course of the nucleolar and nucleoplasmic RNA polymerase reactions in isolated brain nuclei from 11-day chick embryos. The assay of the 2 RNA polymerase reactions in the presence (○) or in the absence (●) of S-100 (100 µg/ml) was as described in the Table. *A*, Mg^{2+}-activated RNA polymerase reaction; *B*, Mn^{2+}-$(NH_4)_2SO_4$-activated RNA polymerase reaction.

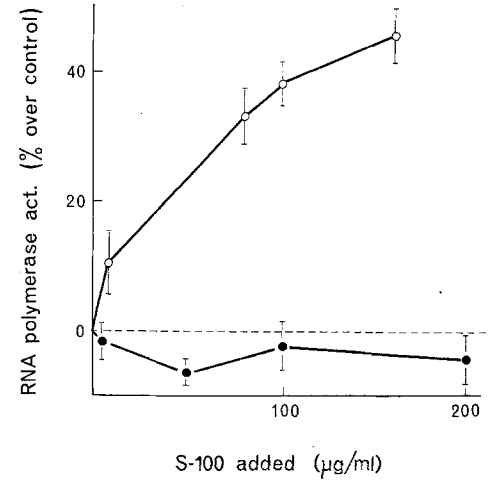

Fig. 2. Effect of various concentrations of S-100 protein on the nucleolar and nucleoplasmic RNA polymerase reactions in isolated brain nuclei from 11-day chick embryos. The experimental details were as described in the Table. S-100 protein was added at the concentrations indicated both in the preincubation and reaction mixtures. ○–○ Mg^{2+}-activated RNA polymerase reaction; ●–● Mn^{2+}-$(NH_4)_2SO_4$-activated RNA polymerase reaction. Each symbol is the mean value of 4 experiments ± S.D.

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].

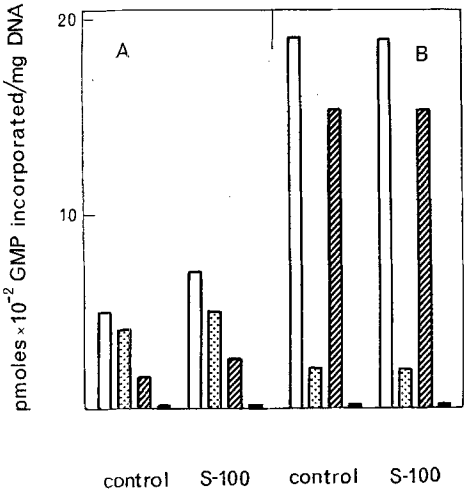

Fig. 3. In vitro effect of α-amanitin and actinomycin D on RNA polymerase activities stimulated by S-100 protein. The activity of the nucleolar (A) and nucleoplasmic (B) RNA polymerase was determined in isolated brain nuclei from 11-day chick embryos as described in the Table. S-100, when present, was 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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