Sympathetic Vasodilatation in the Submaxillary Gland and its Enhancement after Chronic Parasympathetic Denervation

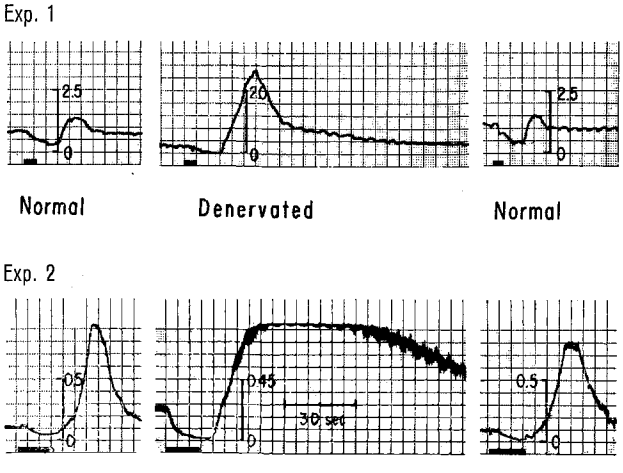
Extensive studies have been made on the parasympathetic vasodilatation in the submaxillary gland[1,2]. It is also well established that, although the main vascular effect of sympathetic nerve stimulation to this gland is vasoconstriction, there is also a consistent after-dilatation which is occasionally very marked, particularly in the cat[3–6]. There is, as yet, no satisfactory explanation for this vasodilatation produced by sympathetic nerve stimulation. CARLSON[7], who first described this effect in 1907, concluded that it was due to sympathetic vasodilator nerve fibres. BARCROFT[8] was of the view that the vasodilatation was secondary to metabolic activity of the gland and that it was effected by some unknown metabolic mediator. HILTON and LEWIS[3] later concluded that the specific metabolic mediator was kallikrein, a potent vasodilator which is present in large amounts in the cells of this gland. Recently, from studies on the perfused submaxillary gland of the cat, others[9] came to the same conclusion, viz., that noradrenaline, and by inference, sympathetic nerve stimulation, causes vasodilatation via the release of kallikrein from the secretory cells. Our present experiments are not consistent with this interpretation, and also present new observations related to the sympathetic vasodilatation after chronic parasympathetic denervation.

Methods. Cats weighing 2.7–4.9 kg were used. Anaesthesia for section of the chorda lingual nerve was carried out with sodium pentobarbital (40 mg kg^{-1} i.p.). The chorda lingual nerve was exposed close to the skull, sectioned and stripped, so that 1–2 cm of it and the chorda tympani nerve were removed. The animal was allowed to recover for 11–23 days (mean 15.2) when the acute experiment was carried out under chloralose anaesthesia (60 mg/kg^{-1} i.v.) after induction with chloroform. Nerve stimulation, blood pressure and injection of drugs were carried out in the acute experiments as described previously[4,5]. Blood flow was measured via a forced convection flow-meter with a probe in an external jugular vein[10] after tying all veins draining into it except that from the gland. Heparin (5 mg/kg^{-1} i.v.) was given prior to insertion of the probe and repeated if necessary. Synthetic bradykinin, and the nonapeptide bradykinin potentiator, (PCA-Trp-Pro-Arg-Gln-Ileu-Pro-Pro) or BPF, were obtained from Schwarz Bioresearch Inc. Atropine was used as the sulphate.

Results and discussion. We have made the following observations on the sympathetic after-dilatation in the submaxillary gland of the cat: 1. It is unaffected by doses of synthetic bradykinin potentiating factor (BPF)[11,12] which greatly augment the vasodilator effects of bradykinin. 2. It is increased after degenerative nerve section of the parasympathetic nerve supply.

Sympathetic vasodilatation in the cat treated with bradykinin potentiating factor (BPF). The BPF nonapeptide was injected i.v. (1–2 mg/kg) in 6 experiments. In every instance this resulted in a marked increase, both in the systemic hypotensive effect and in the local glandular vasodilatation produced by the i.v. injection of bradykinin. The BPF-treated animal responded with a fall in blood pressure of approximately 30 to 50 mm Hg to 1/10 a dose of i.v. injected bradykinin, which was subthreshold before treatment with potentiator. The hypotensive effect of suprathreshold doses of bradykinin was greatly increased and prolonged as previously reported[12]. Nonetheless, in no instance did treatment with BPF increase the sympathetic after-dilatation in the submaxillary gland. Also, on no occasion was there evidence of the local release of bradykinin on nerve stimulation, since a concomitant or subsequent systemic hypotension was never observed. This is in contrast to the systemic overflow of acetylcholine which is readily demonstrated on stimulation of the parasympathetic nerve to this gland in the eserinized cat[12]. We conclude, therefore, that the sympathetic vasodilatation is not mediated by salivary kallikrein or kinin.

Sympathetic vasodilatation after degenerative section of the parasympathetic nerve. Experiments were successfully carried out in measuring blood flow in both submaxillary glands of 9 cats, 11–28 days (mean, 15.2) after section of the chorda lingual nerve on one side. In all experiments except one (28 days denervated), the denervated gland showed a greater after-dilatation than the normal one on stimulation of the sympathetic nerve in



Blood flows through submaxillary glands in 2 separate experiments showing comparison of chronic parasympathetically denervated side to sympathetic nerve stimulation with the contralateral normal side. In each experiment stimulation of the chronically denervated gland is preceded and followed by stimulation of the normal one. Calibrated vertical bars show blood flow in ml min^{-1}; solid horizontal bars indicate period of sympathetic nerve stimulation. Experiment 1. Cat 2.9 kg, 19 days after section of chorda lingual nerve. Contralateral chorda lingual nerve cut at time of acute experiment. Experiment 2. Cat 3.6 kg, 14 days after section of chorda lingual nerve. Contralateral chorda lingual nerve intact at acute experiment.

[1] A. S. V. BURGEN and N. G. EMMELIN, *Physiology of the Salivary Glands* (Ed. Arnold, Ltd. London 1961), p. 127.
[2] M. SCHACHTER, *Handbook of Experimental Pharmacology* (Springer-Verlag, Heidelberg 1970), p. 400.
[3] S. M. HILTON and G. P. LEWIS, J. Physiol., Lond. *134*, 471 (1956).
[4] K. D. BHOOLA, J. MORLEY, M. SCHACHTER and L. H. SMAJE, J. Physiol., Lond. *179*, 172 (1965).
[5] M. SCHACHTER and S. BEILENSON, Fedn. Proc. *27*, 73 (1968).
[6] S. BEILENSON, M. SCHACHTER and L. H. SMAJE, J. Physiol., Lond. *199*, 303 (1968).
[7] A. J. CARLSON, Am. J. Physiol. *19*, 408 (1907).
[8] J. BARCROFT, *The Respiratory Function of the Blood*, 1st edn. Cambridge University Press 1914, p. 140.
[9] K. M. GAUTVIK, M. KRIZ and K. LUND-LARSEN, Acta physiol. scand. *86*, 289 (1972).
[10] E. KARPINSKI, Ph. D. Thesis, University of Alberta (1971).
[11] R. J. FREER and J. M. STEWART, Cienc. Cult., S. Paulo *23*, 539 (1971).
[12] M. SCHACHTER, S. BARTON and E. KARPINSKI, Experientia, *29*, 973 (1973).

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.

M. SCHACHTER, SUSANNE BARTON and E. KARPINSKI[14]

The Department of Physiology, University of Alberta Edmonton 7 (Alberta, Canada), 12 June 1973.

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

[14] This work was supported by grants from the Medical Research Council of Canada and the Alberta Heart Foundation.

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

[1] B. W. MOORE, Biochem. biophys. Res. Commun. *19*, 739 (1965).
[2] L. LEVINE and B. W. MOORE, Neurosci. Res. Prog. Bull. *3*, 18 (1965).
[3] B. W. MOORE, V. J. PEREZ and M. J. GEBRING, J. Neurochem. *15*, 265 (1968).
[4] D. KESSLER, L. LEVINE and G. FASMAN, Biochemistry *7*, 758 (1968).
[5] T. J. CICERO, W. M. COWAN, B. W. MOORE and V. SUNTZEFF, Brain Res. *18*, 25 (1970).
[6] N. MIANI, G. DE RENZIS, F. MICHETTI, S. CORRER, C. OLIVIERI SANGIACOMO and A. CANIGLIA, J. Neurochem. *19*, 1387 (1972)
[7] E. DE ROBERTIS, Science *156*, 907 (1967).
[8] J. E. ZUCKERMAN, H. R. HERSCHMAN and L. LEVINE, J. Neurochem. *17*, 247 (1970).
[9] P. CALISSANO and A. D. BANGHAM, Biochem. biophys. Res. Commun. *43*, 504 (1971).
[10] H. HYDÉN and P. W. LANGE, Expl Cell Res. *62*, 125 (1970).
[11] F. MICHETTI, N. MIANI, G. DE RENZIS, A. CANIGLIA and S. CORRER, *Proteins of the Nervous System* (N.A.T.O. Advanced Study Institute, Cortona 1972).
[12] N. MONTANARO, F. NOVELLO and F. STIRPE, Biochem. J. *125*, 1087 (1971).