while growth hormone (an anabolic hormone) increases REM sleep in rats[15] and cats[16]. In sum, our experiments suggest that proteins may be importantly related to sleep and in particular to REM.

*Résumé.* Des chats auquels on a implanté stereotaxiquement un système de cannules «push-pull» dans la formation reticulée mésencephalique ont été soumis à des périodes de perfusion pendant la veille ou pendant la phase REM du sommeil. Les expériences ont démontré que la phase REM est accompagnée d'une augmentation très significative ($p < 0.0001$) des proteines en comparaison avec la veille. Ces résultats sont discutés par rapport au rôle possible de la synthèse des protéines pendant la phase de sommeil.

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[16] W. C. STERN, J. C. JALOWIEC, H. SHABSHALOWITZ and P.J. MORGANE, Hormones Behav., in press, 1975.

## Potentiating Effect of β-Adrenergic Stimulant on the Response of the Cutaneous Vascular Resistance to α-Adrenergic Stimulant

It is well known that a stimulation of adrenergic β-receptors causes the relaxation of vascular smooth muscles, particularly in the skeletal muscle and coronary arteries which have relatively increased myogenic tone. On the other hand, the myogenic tone of the cutaneous resistance vessels is relatively decreased and a stimulation of their adrenergic β-receptors does not necessarily produce the relaxation. Furthermore, the cutaneous artery is one of the arteries which have the most sensitive response to vasoconstrictor agents in the body. Recently, many investigators have reported that the β-receptor adrenergic blocking agents have hypotensive action in hypertensive patients[1–3]. It is, therefore, of interest to elucidate the pharmacological characteristics of the adrenergic β-receptors in the resistance vessels.

The purpose of this paper is to evaluate an effect of β-adrenergic stimulation on the response to catecholamines (CA) using the isolated rabbit ear, which consists almost entirely of the cutaneous vascular beds. Male rabbits weighing 2.0 to 3.0 kg were anesthetized with sodium isomital (1.0 mg/kg i.v.) following heparin injection (1000 U/kg i.v.). The central arteries of bilateral ears were cannulated with fine polyethylene tubing and about 1 cm of cannula was inserted into them, then the ears were removed at their bases. They were stored at 3–4 °C in normal Krebs bicarbonate solution for 24 to 48 h. Under these conditions, the degeneration of sympathetic nerves had occurred and their function had failed[4]. The central arteries were perfused with normal Krebs bicarbonate solution, equilibrated with a gas mixture of 95% $O_2$ + 5% $CO_2$, at 37 °C by means of a roller pump delivering a constant rate of flow (1.25 ml/min). This solution had the following composition, in m$M$: $Na^+$ 162.0, $K^+$ 5.9, $Ca^{++}$ 2.5, $Mg^{++}$ 1.25, $Cl^-$ 150.4, $HCO_3^-$ 25.0 and glucose 8.3 (pH 7.4). Prior to each experiment, perfusion was always made for 2 h in order to obtain a constant condition. Changes in the vascular resistance were recorded on the kymograph as changes in perfusion pressure with a mercury manometer. 0.1 ml of suitable concentrations of CA, freshly prepared in normal Krebs solution, was injected intraarterially through a rubber tube connected close to the central arterial cannula. To observe an effect of the drugs on the response to CA, the perfusion was performed with normal Krebs solution containing their suitable concentrations for 30 min, and then the responses were compared between those before and during the perfusion.

In the present experiment, the relative potencies of adrenaline (A) (0.1 μg), noradrenaline (NA) (0.1 μg), methoxamine (MX) (0.1 μg) and MX (1.0 μg) were approximately 2.0: 1.0: 0.3: 0.5. In comparison with NA (0.1 μg), the response to A (0.1 μg) was significantly greater ($p < 0.01$), and the responses to 0.1 μg and 1.0 μg of MX were significantly smaller ($p < 0.01$). In the presence of L-isoproterenol (IP) of $10^{-10}$ to $10^{-6}$ g/ml, perfusion pressure did not change. As shown in Figure 1, IP ($2 \times 10^{-10}$ g/ml) potentiated the responses to both NA and MX. It was reported that a few min after the stimulation of the adrenergic β-receptors, cyclic adenosine 3′, 5′-monophosphate (cyclic AMP) levels in the vascular smooth

[1] B. N. C. PRICHARD and P. M. S. GILLAM, Brit. med. J. *1*, 7 (1969).
[2] F. J. ZACHARIAS, K. J. COWEN, J. PRESTT, J. VICKERS and B. G. WALL, Am. Heart J. *83*, 755 (1972).
[3] J. A. FRANCIOSA, E. D. FREIS and J. CONWAY, Circulation *48*, 118 (1973).
[4] L. B. GEFFEN and C. C. HUGHES, J. Physiol., Lond. *221*, 71 (1972).

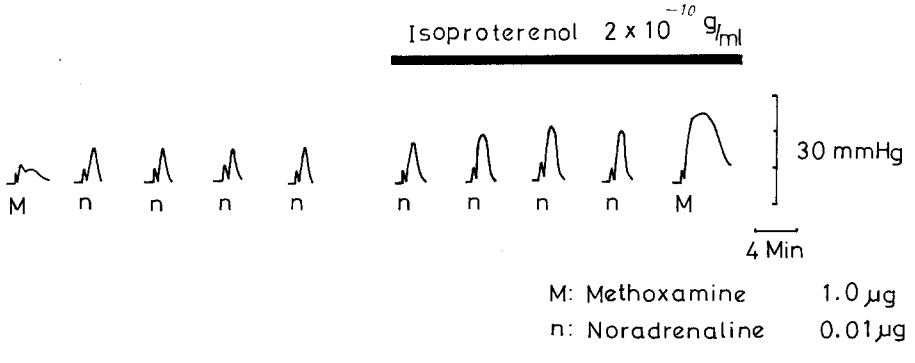


Fig. 1. Effect of L-isoproterenol on the responses of peripheral vascular resistance to catecholamines. Noradrenaline was repeatedly applied every 15 min.

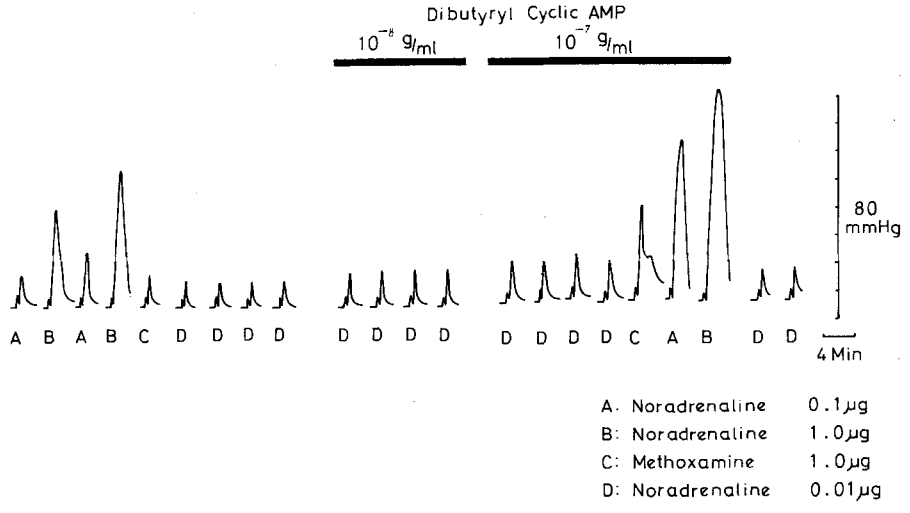


Fig. 2. Effect of dibutyryl cyclic AMP on the responses of peripheral vascular resistance to catecholamines. Noradrenaline was repeatedly applied every 15 min.

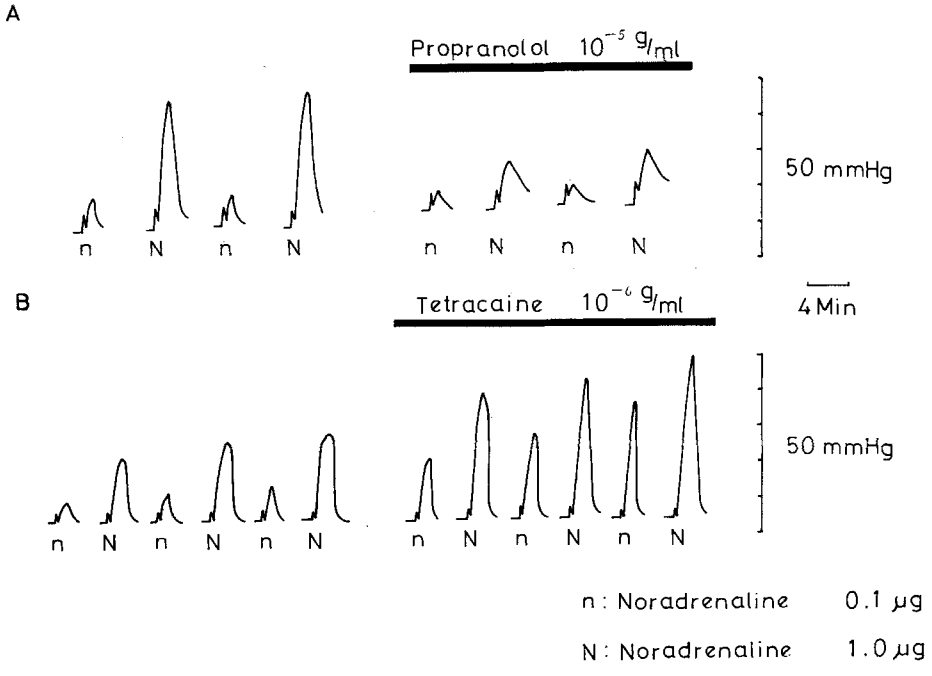


Fig. 3. Effect of propranolol (A) and tetracaine (B) on the responses of peripheral vascular resistance to noradrenaline. Noradrenaline was repeatedly applied every 15 min.

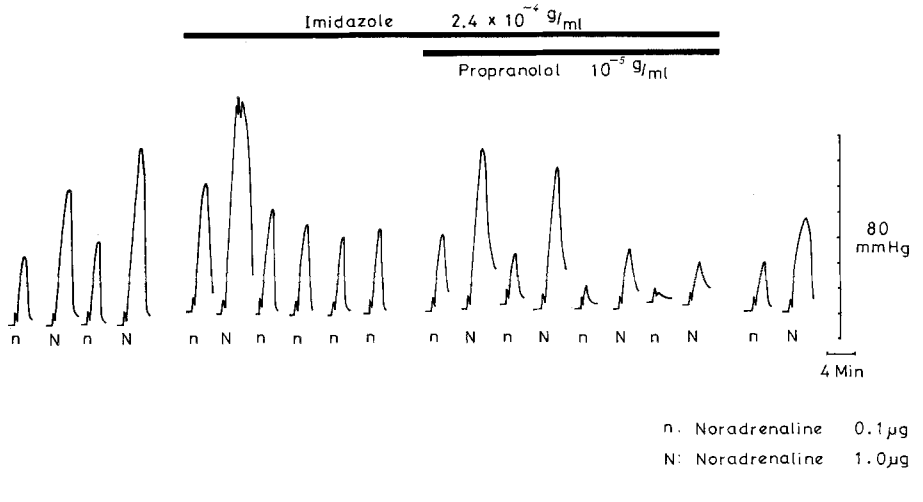


Fig. 4. Effects of both imidazole and propranolol on the responses of peripheral vascular resistance to noradrenaline. Noradrenaline was repeatedly applied every 15 min.

muscle cells were increased[5]. Hence, the effect of dibutyryl cyclic AMP, permeable to the muscle cell membrane, on the responses to CA was examined, and it was observed that dibutyryl cyclic AMP ($10^{-7}$ to $10^{-8}$ g/ml) augmented the responses to both NA and MX (Figure 2).

Propranolol ($10^{-5}$ g/ml) inhibited the response to NA and, in contrast, it was potentiated with tetracaine ($10^{-6}$ g/ml) (Figure 3). Therefore, the inhibitory action of propranolol may be caused not by a local anesthetic action, but by a $\beta$-blocking one. And so there is a possibility that the inhibitory effect of propranolol is mediated by its suppressive action on CA to increase cyclic AMP concentration in the smooth muscle cells[5,6]. When 0.1 $\mu$g and 1.0 $\mu$g of NA were applied repeatedly and alternately every 15 min, the responses were augmented gradually. In the presence of imidazole ($2.4 \times 10^{-4}$ g/ml), a phosphodiesterase activator, the response to NA was enhanced initially, then its gradual increase ceased and finally reached an almost constant level. An application of propranolol ($10^{-5}$ g/ml), in addition to imidazole, strongly inhibited the response (Figure 4). On the other hand, caffeine, a phosphodiesterase inhibitor, potentiated the response to NA in lower concentration ($2 \times 10^{-7}$ g/ml), while its higher concentration ($9.7 \times 10^{-4}$ g/ml) suppressed them.

The results described above show that adrenergic $\beta$-stimulant potentiates the action of adrenergic $\alpha$-stimulant, since the response to NA is increased in the presence of IP or dibutyryl cyclic AMP. The stimulating effect of adrenergic $\alpha$-receptor, therefore, appears to be more marked, when the stimulation of $\beta$-receptor does coexist. Since, in the cutaneous resistance vessels, adrenergic receptors mainly consist of $\alpha$-receptor, not of $\beta$-receptor, a relaxation of the vessels by stimulation of $\beta$-receptor hardly occurs. As mentioned above, dibutyryl cyclic AMP augmented the responses to NA and MX. Therefore, it is suggested that the inhibitory action of propranolol may be due to the suppression of the increase in cyclic AMP levels. This insight may be supported by the observation that an increase in tissue content of cyclic AMP potentiates the contraction of the rat's aortic strip produced by NA[7]. The aortas of spontaneously hypertensive rats (SHR) contained significantly lower intracellular concentrations of cyclic AMP then their controls, and the response of adenyl cyclase activity to IP was reduced, while phosphodiesterase activity was significantly elevated[8]. Therefore, regarding adrenergic receptors and adenyl cyclase system, it is speculated that a similar relationship exists between the aorta of SHR and the cutaneous resistance vessels of rabbit ear, and such characteristics may be associated with an increase in the vasoconstrictor response to NA in the artery of SHR[9].

*Zusammenfassung.* Die Wirkung von Noradrenalin auf den peripheren vaskulären Widerstand wurde am isolierten Kaninchenohr untersucht und festgestellt, dass die Wirkung einer $\alpha$-adrenergen Stimulation durch $\beta$-adrenerge Stimulation verstärkt wird.

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[5] L. TRINER, G. G. NAHAS, Y. VULLIEMOZ, N. I. A. OVERWEG, M. VEROSKY, D. V. HABIF and S. H. NGAI, Ann. N.Y. Acad. Sci. *185*, 458 (1971).
[6] R. ANDERSSON, Acta physiol. scand. *87*, 84 (1973).
[7] H. J. BARTELSTONE, P. A. NASMYTH and J. M. TELFORD, J. Physiol., Lond. *188*, 159 (1967).
[8] M. S. AMER, Science *179*, 807 (1973).
[9] B. FOLKOW, M. HALLBÄCK, Y. LUNDGREN and L. WEISS, Acta physiol. scand. *80*, 93 (1970).

## Resting Membrane Potential of the Stria Cells of the Guinea-Pig

The object of this paper is to examine the effect of potassium ions on the resting membrane potential of the stria cells. A higher concentration of potassium ions in the external environment of muscle cells or nerve cells has been known to depolarize the resting membrane potential[1-3]. The present study was undertaken because the stria cells face the endolymph, and the endolymph contains a high concentration of potassium ions (150 mEq/l)[4], and the resting membrane potentials have not been previously measured, although BÉKÉSY[5-7] did report that inside the stria cells the potential was negative, but gave no measurements.

*Method. Electrode.* Fine microglass electrode with tip diameter between 0.5 $\mu$m to 0.8 $\mu$m were used to record the endocochlear DC potential from the scala media and the resting membrane potential of the stria cells. The electrodes were filled with 3 *M* KCl. The resistances were checked before using, only those in the range between 15 $M\Omega$ to 30 $M\Omega$ and with low tip potential (between 5–8 mV) were selected for measurements. A high input impedance differential electrometer amplifier Keithley 604 or Nihon Koden microglasselectrode amplifier was used for recording.

*Measurements of resting membrane potentials in vivo.* Coloured and white guinea-pigs were used throughout this study. The animal was deeply anaesthetized under Nembutal, and the head was firmly fixed on a headholder. The bulla was opened as previously described[8].

A small hole (diameter about 50–80 $\mu$m) just above the middle region of the stria-ligamentum was made on the bony cochlear wall by means of a fine steel-needle which bare 3 sharpened edges. Care was taken to avoid bleeding.

The Ag-AgCl-microglass electrode was adjusted to a 90° direction so that it could pass into the hole and through the cells of ligamentum spirale and the 3 layered cells of stria vascularis. The insertion of the electrode with the aid of a Leitz manipulator was advanced gently in order to show the clear potential drop negative potential just before the registration of the endocochlear DC potential. This negative potential probably represented the resting membrane potential of the stria cells in vivo.

[1] A. L. HODGKIN and B. KATZ, J. Physiol., Lond. *108*, 37 (1949).
[2] A. F. HUXLEY and R. STAMPFLI, J. Physiol., Lond. *112*, 496 (1951).
[3] G. LING and R. W. GERARD, J. comp. Cell Physiol. *34*, 383 (1949).
[4] C. A. SMITH, O. H. LOWERY and M.-L. WU, Laryngoscope, St. Louis *64*, 141 (1954).
[5] G. v. BÉKÉSY, Nature, Lond. *169*, 241 (1952).
[6] G. v. BÉKÉSY, J. acoustic Soc. Am. *24*, 72 (1952).
[7] G. v. BÉKÉSY, *Experiments in Hearing* (McGraw and Hill, New York 1962).
[8] J. T.-Y. CHOU, Arch. Ohr.-Nas.-u. KehlkHeilk. *195*, 246 (1970).