EFFECTS OF CATECHOLAMINES ON SUPRA-OPTIC NUCLEUS NEURONES IN ORGAN CULTURE*

BERNARD H. MARKS, KAKUICHI K. SAKAI, JACK M. GEORGE and ADALBERT KOESTNER,
Departments of Pharmacology, Medicine and Veterinary Pathobiology,
The Ohio State University, Columbus, Ohio 43210, U.S.A.

STUDYING the effects of drugs upon neuro-endocrine systems has been a highly inexact science, largely frustrated by problems of experimental design. Thus for the most part, with the techniques currently available, it has not been possible to know the effective drug concentrations at the presumed site of drug action, to be certain whether a given drug is acting directly upon a neuro-secretory cell or at some upstream site, or even to know whether applied drugs are acting presynaptically or post-synaptically upon neuro-endocrine units. In order to avoid these problems, and permit more direct study of drug effects upon receptor mechanisms associated with neuro-endocrine functions, we have developed an isolated organ-cultured neuro-endocrine test system, in which the neural units are devoid of efferent connections. The system is the organ-cultured supra-optic nucleus derived from the brain of newborn puppies.

The method consists in dissecting, mincing and explanting on to coverslips the supra-optic nuclei from young pups (KOESTNER *et al.*, 1972). The explants are cultured in Leighton tubes with small volumes of a medium containing balanced salts, bovine fetal serum, bovine adult serum ultrafiltrate and glucose. The medium is changed every 3 days. Under these conditions, death and degeneration of damaged cells is complete in 7–10 days, the remaining supra-optic neurones remaining viable and growing out new axons. Study of the adrenergic receptor mechanisms of these cells were carried out after 2–3 weeks of culture.

Cultured cells were studied after transferring each coverslip bearing an explant to a small warmed (37°C) chamber, volume 0·5 ml, filled with Kreb's–Henseleit medium and gassed with 95% O_2–5% CO_2. By controlled gravity flow and suction, oxygenated K–H medium or drugs dissolved in K–H medium was perfused through the chamber. Under a dissecting microscope, individual neurones in each explant could be penetrated with conventional glass micro-electrodes in order to record resting and action potentials.

Using this method, a resting membrane potential of $-36 \pm 0{\cdot}69$ mV was recorded from 112 cells. The majority of these cells were quiescent, with only rare spontaneous action potentials. Continuous nerve activity could be generated by perfusion either with 10^{-6} M glutamate or nicotine. Spikes generated by such treatment had an approximate amplitude of 45 mV, and, as illustrated in Fig. 1A, showed a spike duration of approximately 6 msec with a characteristic negative afterpotential that had a very much longer duration.

When either norepinephrine, isoproterenol or phenylephrine was perfused through

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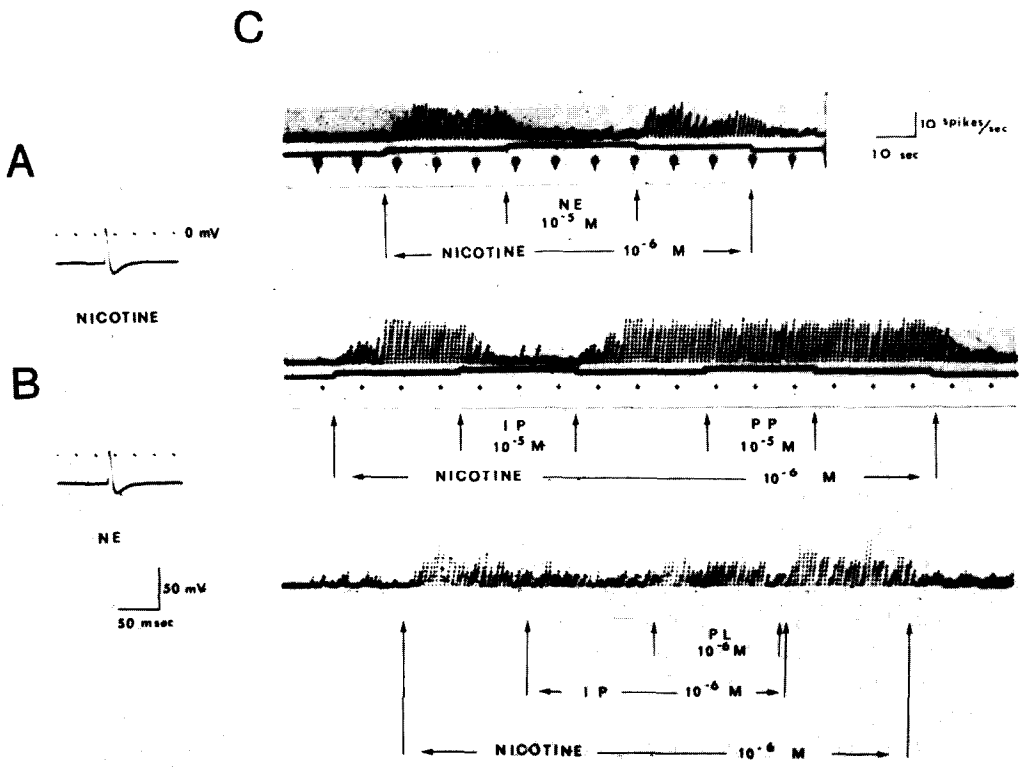

FIG. 1.—Effects of catecholamines on action potential configuration and on spike frequency recorded from supra-optic nucleus neurones in organ culture. A, spike configuration during exposure to nicotine 10^{-6} M. B, spike configuration during exposure to nicotine plus norepinephrine (NE) 10^{-6} M. C, inhibition of nicotine-induced supra-optic neurone spiking activity by NE (upper tracing), by isoproterenol (IP-middle tracing) and absence of effect of phenylephrine (PP-middle tracing); reversal of the effect of isoproterenol by propranolol (PL-lower tracing).

the explants, no change could be seen either in resting potential or in the frequency of the occasional spontaneous action potentials. Therefore, the effect of catecholamines was examined on cultures in which a high spike frequency was generated by continuous exposure either to glutamate or to nicotine 10^{-6} M. Under these conditions concentration-dependent inhibition of spike frequency is produced by concentrations of norepinephrine between 10^{-7} to 10^{-4} M. The upper tracing of Fig. 1C illustrates the inhibition of nicotine spiking produced by exposure to norepinephrine. Exactly the same norepinephrine response is observed if glutamate is used to initiate membrane activity. It is noteworthy that, during the period in which norepinephrine is inhibiting spiking activity, the configuration of those action potentials which do occur is not significantly different from the action potentials that develop during the control period with nicotine (Fig. 1B) or with glutamate. Only the frequency of spike generation is altered. This is in contrast to the effect of gamma-amino butyric acid (GABA), which also reduces the spike frequency with a concentration dependence much like that of norepinephrine. However, the individual spikes recorded during exposure to GABA

are found to have a more negative resting potential and the duration of the negative afterpotential is seen to be markedly prolonged by GABA. Thus, one might infer that the ionic basis for the inhibition of nerve spiking activity is different for norepinephrine and for GABA, though the final effect on spike frequency is the same.

In cells in which high frequency spiking is induced by nicotine or by glutamate, it is possible to identify the nature of the catecholamine receptor mechanism mediating the inhibitory effect of norepinephrine. The middle tracing of Fig. 1C illustrates one such approach, showing that the inhibitory effect of norepinephrine is also readily demonstrated by perfusion with isoproterenol, but no effect on spike frequency is observed when cultured cells are exposed to phenylephrine. The lower tracing of Fig. 1C demonstrates that the inhibitory effect of isoproterenol on spike frequency can be reversed by exposure of the cultures to propanolol. Thus, the receptor mechanism in the organ-cultured supra-optic nucleus neurones is apparently of the beta type.

These experiments suggest that it may be feasible to use the isolated organ-cultured supra-optic nucleus as a test system to study the relationship between cell membrane receptor activation and the subsequent processes involved in the function of typical neuro-endocrine units. These explants, after culture for weeks, appear to behave exactly as they do *in situ*, insofar as their response to cholinergic and adrenergic agonists and antagonists are concerned (BARKER *et al.*, 1971). These responses are consistent with the effects of nicotine, acetylcholine and norepinephrine upon vasopressin secretion (BURN *et al.*, 1945; ABRAHAMS and PICKFORD, 1956), and with the known occurrence of acetylcholine and norepinephrine in the supra-optic nucleus (FELDBERG and VOGT, 1948; CARLSSON *et al.*, 1962). Thus, it would appear to be valid to use this isolated neural system to inquire into the processes that regulate the sensitivity of these membrane receptors, and that translate receptor activation into neurosecretion—the fundamental process that transduces neural into endocrine information.

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