the ejecting current required to evoke equivalent responses, and in terms of the magnitude of the responses evoked by identical ejecting pulses. However, this apparent difference in potency might be due to a difference between the physical mobilities of the two drugs.

Excitatory responses to both DOPA and NA could be reversibly antagonized by phentolamine (12 cells). Excitatory responses to DOPA and NA could also be antagonized by propranolol (6 cells); responses to acetylcholine were not affected. On the other hand, excitatory and depressant responses to DOPA were not affected by atropine applied with ejecting currents sufficient to antagonize excitatory responses to acetylcholine (6 cells).

These results show that cortical neurones are sensitive to locally administered DOPA, and suggest that DOPA may activate receptors similar to those activated by NA. However, it is not clear from the present results whether DOPA acts directly on post-synaptic receptors, or indirectly via the release of NA from pre-synaptic terminals.

This work was supported by the Mental Health Trust and Research Fund.

References

ANDÉN, N.-E., CARLSSON, A. & HÄGGENDAL, J. (1969). Adrenergic mechanisms. *Ann. Rev. Pharmac.*, 9, 119-134.

BEVAN, P., BRADSHAW, C.M., ROBERTS, M.H.T. & SZABADI, E. (1974). The effect of microelectrophoretically applied mescaline on cortical neurones. *Neuropharmacology*, 13, 1033-1045.

FUXE, K. (1965). Evidence for the existence of monoamine neurones in the central nervous system. IV Distribution of monoamine nerve terminals in the central nervous system. *Acta Physiol. Scand.*, 64, suppl. 247.

HORNYKIEWICZ, O. (1974). The mechanisms of action of L-DOPA in Parkinson's disease. *Life Sciences*, 15, 1249-1259.

Induced receptors for noradrenaline and serotonin in guinea-pig vas deferens?

R.J. REIFFENSTEIN* & E. WOYTIUK

Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada

We have previously reported recovery of responsiveness to noradrenaline during exposure to methacholine in tissues whose α-adrenoceptors were completely blocked by phenoxybenzamine (Iijima & Reiffenstein, 1972). Methacholine could also induce serotonin receptors. Further investigation of these induced receptors is reported here. Stripped guinea-pig vas deferens was mounted in Krebs solution at 37°C and isotonic contractions obtained under 0.5 g tension. Agonist concentrations used throughout were maximal. Induced serotonin receptors were investigated in normal tissues, while induced α-adrenoceptors were studied in tissues pretreated with phenoxybenzamine until response to noradrenaline was eliminated. Contractions were then obtained to serotonin, or noradrenaline, in the presence of a receptor-inducing agent (methacholine and others). To investigate whether noradrenaline and serotonin might be reacting with the same receptor, comparative blocking studies were done by cumulative additions of antagonists during responses to combined inducer (methacholine) and agonist. The induced α-adrenoceptors were as susceptible to phentolamine ($ID_{50} = 1.2 \times 10^{-5}$ M *vs.* noradrenaline) as were normal α-adrenoceptors ($ID_{50} = 2 \times 10^{-5}$ M). Methysergide (up to 10^{-3} M) failed to inhibit induced responses to noradrenaline. The induced response to serotonin could be blocked by phentolamine ($ID_{50} = 1.8 \times 10^{-4}$ M) but lower concentrations of methysergide were effective ($ID_{50} = 3 \times 10^{-5}$ M). Thus it is likely that two distinct receptors were involved. Agents other than methacholine also proved capable of reviving responsiveness to noradrenaline: KCl (1×10^{-2} M), $BaCl_2$ (2×10^{-8} M), or propranolol (2×10^{-4} M). The latter has been observed to have a similar effect in vasculature (Yamamura & Horita, 1968; Janis & Triggle, 1974). By themselves, these agents produced widely different contractions (or none at all) so that contraction does not seem to be a requirement. Should the substances, such as methacholine or propranolol, producing this effect depolarize the smooth muscle and thereby reduce the threshold for spike generation, then previously subthreshold receptor activation could then produce contraction and there would be no need to postulate the induction of new receptors. The revived responses to noradrenaline were also potentiated by cocaine, but cocaine had no effect in the absence of the receptor-inducing agent (when noradrenaline itself produced no effect). This supports the contention of Nakatsu & Reiffenstein (1968) that cocaine increases maximal responses to noradrenaline by increasing the efficacy of unblocked receptors rather than uncovering phenoxybenzamine-inhibited receptors. We also confirmed our original

observation that the induced α-adrenoceptors were insensitive to phenoxybenzamine. The tissues were re-exposed to phenoxybenzamine in the presence of methacholine (which would be expected to protect the muscarinic receptors, and maximally induce α-adrenoceptors). Responses to noradrenaline were only reduced in proportion to the responses to methacholine. This suggests that methacholine is temporarily uncovering or unblocking the phenoxybenzamine-inhibited α-adrenoceptors. A corollary is that phenoxybenzamine does not alkylate directly to the α-adrenoceptor. This phenomenon could not be duplicated in guinea-pigs from the U.K. since their vasa deferentia do not contract in response to noradrenaline.

Supported by Medical Research Council of Canada (MA 4979).

References

IIJIMA, A.T. & REIFFENSTEIN, R.J. (1972). Possible allosteric interactions between pharmacological receptors in guinea-pig vas deferens. *Br. J. Pharmac.*, 44, 364P-365P.

JANIS, R.A. & TRIGGLE, D.L. (1974). On the reversal by (+)- and (−)-propranolol of irreversible α-adrenoceptor blockade in rabbit aorta. *Pharm. Res. Comm.*, 6, 55-60.

NAKATSU, K. & REIFFENSTEIN, R.J. (1966). Increased receptor utilization: mechanism of cocaine potentiation. *Nature, Lond.*, 217, 1276-1277.

YAMAMURA, H.L. & HORITA, A. (1968). Effect of propranolol on the blockade of *alpha* adrenergic receptors. *J. Pharmac. exp. Ther.*, 164, 82-89.

The effect of chlorpromazine on carbachol binding to muscarinic receptors in intestinal smooth muscle

ELAINE HAIGH & J.M. YOUNG*

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD

The binding of agonists to muscarinic receptors in strips of longitudinal muscle from guinea-pig small intestine, as measured by the inhibition of the binding of an affinity label for the muscarinic receptor, [^{3}H]-propylbenzilylcholine mustard ([^{3}H]-PrBCM), differs from that of antagonists. Whereas antagonist binding curves in general approximate well to those expected for a simple mass-action interaction, those for agonists show an apparent negative co-operativity (Young, 1974). Similar effects have been observed using a microsomal fraction (Burgen & Hiley, 1975). There is other evidence that the binding of agonists differs from that of antagonists. Exposure of muscle strips to distilled water prior to the usual 1 h preincubation in normal Krebs solution results in a shift of the binding curve for carbachol to the left without any apparent change of slope, whereas the curve for methylatropinium bromide is unaltered (Taylor & Young, unpublished observations). What structural element is modified by the hypotonic treatment leading to the long-lasting changes in carbachol binding, and in particular whether this is an effect on the receptor macromolecule or its supporting membrane, is unknown. In an attempt to explore the relationship between membrane structure and receptor conformation we have examined the effect of chlorpromazine, a drug whose membrane actions have been extensively studied (Seeman, 1972).

Chlorpromazine, added 30 min prior to the mustard and present throughout, acted as a competitive inhibitor of [^{3}H]-PrBCM binding ($K_a = 1.4 \times 10^6$ M^{-1}), in agreement with Miller & Hiley (1974). However, treatment of the muscle strips for 30 min at room temperature (22°) with higher concentrations of chlorpromazine ($10^{-5} - 10^{-4}$ M), corresponding to the prelytic-lytic range on erythrocytes, resulted in a decrease in [^{3}H]-PrBCM binding measured after 60 min washing in Krebs solution alone. Atropine (10^{-6} M) did not prevent this long-lasting effect of chlorpromazine. The loss of binding was apparently not due to solubilization of receptor material since 10^{-4} M chlorpromazine failed to remove the tritium from strips previously labelled with [^{3}H]-PrBCM. The curve for the inhibition by methylatropinium bromide of [^{3}H]-PrBCM binding to strips pretreated with 2×10^{-5}M chlorpromazine was unaltered from that to normal strips (best fit parameters: Hill slope, 0.99; K_a, 1.2×10^8 M^{-1}; non-specific binding of [^{3}H]-PrBCM, 13%). In contrast the mean Hill slope of the binding curve deduced for carbachol was increased from 0.4 in normal strips to 0.6 in chlorpromazine pretreated strips, with a small increase in the ED_{50}. Thus pretreatment of longitudinal muscle strips with prelytic concentrations of chlorpromazine has an effect on the binding curve for carbachol similar to that of prior desensitization.