The reduction in alpha$_1$ adrenoceptor mediated [^{32}P]phosphatidylinositol turnover following prolonged exposure to noradrenaline is consistent with the observed reduction in receptor concentration. Increases in [^{32}P]phosphatidylinositol turnover due to alpha$_1$ adrenoceptor activation have been postulated to be secondary, in response to an increased rate of phosphatidylinositol hydrolysis by phospholipase C [18]. Harrington and Eichberg [19] have recently demonstrated the breakdown of phosphatidylinositol in a reconstituted cell-free preparation by an apparently alpha$_1$ adrenoceptor mediated mechanism. Whether the reduction in [^{32}P]phosphatidylinositol turnover demonstrated by us represents an inability of alpha$_1$ adrenoceptors to activate phospholipase C either directly or indirectly remains to be determined.

In summary, our experiments indicate that the concentration of alpha$_1$ adrenoceptors on vascular smooth muscle cells in culture is reduced following prolonged exposure to noradrenaline. There appears to be no significant change in the affinity of the remaining receptors for noradrenaline. This reduction in receptor number is accompanied by a reduction in alpha$_1$ adrenoceptor mediated turnover of cell [^{32}P]phosphatidylinositol. The greater magnitude of the reduction in [^{32}P]phosphatidylinositol turnover over the reduction in receptor number suggests that for this cell response there may be (i) spare alpha$_1$ adrenoceptors, or (ii) the process of alpha$_1$ adrenoceptor desensitization involves receptor uncoupling, possibly from phospholipase C as well as receptor loss.

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Noradrenergic inhibition of the nicotinically-stimulated release of acetylcholine from guinea-pig ileal synaptosomes

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Paton and co-workers [1, 2] first demonstrated that electrical stimulation of segments of the guinea-pig ileum produces contractions which are mediated by the release of acetylcholine (ACh) from nerve terminals of the myenteric plexus. Norepinephrine (NE) inhibits the contractions and the release of ACh by a mechanism which involves α-adrenergic receptors [3, 4]. Following Langer's proposal [5] that α-adrenoceptors are divided into the postsynaptic α_1-adrenoceptor and the presynaptic α_2-adrenoceptor, it was shown that the cholinergic activity in the intact ileal myenteric plexus is modulated by α_2-adrenoceptors [6–8].

We have described previously a preparation of synaptosomes derived from the myenteric plexus of the guinea-pig ileum [9]. Both 50 mM KCl and 10 μM 1,1-dimethyl-4-phenyl piperazinium (DMPP) increase the calcium-dependent release of [^{3}H]ACh from these synaptosomes [10], but only the DMPP-induced release is modulated significantly by oxotremorine [10] or adenosine [11]. Based on the ability of NE to inhibit both the electrically-stimulated and nicotinically-induced [12] release of ACh from the intact myenteric plexus, we decided to determine whether NE might modulate the nicotinically-stimulated release of [^{3}H]ACh from the synaptosomal preparation and, if so, to determine what type of receptor was involved.

Methods

Preparation of the P_2 fraction. The P_2 fraction was obtained from the guinea-pig ileum longitudinal muscle-myenteric plexus preparation as previously described [11].

Synthesis and release of [^{3}H]acetylcholine. The P_2 pellet was suspended in 5 ml of Krebs–Ringer bicarbonate buffer of the following composition (mM concentrations): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25 and dextrose, 10. The buffer was gassed with 95% O_2–5% CO_2 to maintain a pH of 7.4. The tissue was

incubated for 30 min at 37° in the presence of 3.0 μM [^{3}H]choline (5.0 10^3 Ci/mole), followed by centrifugation at 5000 *g* for 10 min. After washing the tissue, the pellet was resuspended in the Krebs–Ringer buffer, and portions of the suspension were added to tubes containing the appropriate drugs for a final volume of 220 μl. All assays contained a final concentration of 10 μM physostigmine. When adrenergic antagonists were evaluated, they were added to the tissue 10 min prior to the addition of the tissue to tubes containing the other drugs. The tubes were shaken and incubated for 2 min at 37° under 95% O_2–5% Co_2. The release was terminated by returning the tubes to the ice bath. The tubes were centrifuged at 3600 *g* for 10 min, and the supernatant fractions were collected.

Determination of [^{3}H]acetylcholine. The [^{3}H]ACh was separated from [^{3}H]choline and determined using a previously described modification [9] of the choline kinase-ion pair extraction method of Nemeth and Cooper [13].

Materials. The materials used were: 80 x 10^3 Ci/mole [*methyl*-^{3}H]choline chloride (New England Nuclear, Boston, MA); Liquiscint (National Diagnostics, Somerville, NJ); butyronitrile (Aldrich Chemical Co., Milwaukee, WI); l-epinephrine bitartrate, glycylglycine (Calbiochem-Behring, La Jolla, CA); prazosin hydrochloride (Pfizer, New York, NY); clonidine hydrochloride (Boehringer-Ingelheim, Indianapolis, IN); and adenosine 5′-triphosphate, choline chloride, adenosine 5′-triphosphate:choline phosphotransferase (EC 2.7.1.32; choline kinase), DMPP iodide, tetraphenylboron, l-NE bitartrate, l-phenylephrine hydrochloride, yohimbine hydrochloride, physostigmine sulfate and l-isoproterenol bitartrate (Sigma Chemical Co., St. Louis, MO).

Results and discussion

Effects of α- and β-adrenergic agonists on release of [^{3}H]ACh. The basal release of [^{3}H]ACh from the ileal synaptosomes was reduced only 25% by 10 μM NE (data not shown). The DMPP-induced release of [^{3}H]ACh from these synaptosomes, however, was inhibited significantly by increasing concentrations of both NE and epinephrine (E) (Fig. 1). The dose–response curve of epinephrine was not significantly different from that of NE. The curve of E, however, appears to be somewhat to the left of that of NE, indicating that E may be slightly more potent than NE, as was the case when the resting and electrically-stimulated release of ACh was assessed using the longitudinal muscle-myenteric plexus preparation [3]. The β-adrenergic agonist isoproterenol had no effect on the DMPP-induced release at concentrations up to 10 μM, as was the case with dopamine (data not shown). Drug concentrations above 10 μM were not tested. The finding that isoproterenol was ineffective is in agreement with previous studies of the ACh release from intact preparations [3, 4]. The evidence suggested that the adrenergic receptor modulating the DMPP-induced release was an α-receptor.

Effects of α_1- and α_2-adrenergic agonists on release of [^{3}H]ACh. To determine whether the receptor was of the α_1- or α_2-type [5], the release was assessed in the presence of phenylephrine (α_1), clonidine (α_2) and NE. Clonidine and NE were equipotent inhibitors of the DMPP-induced release (Fig. 2). Wikberg [6] found that clonidine was more potent than NE in its ability to block ileal contractions induced by electrical stimulation of cholinergic neurons. A possible explanation for this difference in potency could be that the nicotinic action and the electrically-stimulated effect might be mediated through different nerve terminals.

Although our results indicated that clonidine inhibited the DMPP-induced release of [^{3}H]ACh, it should be pointed out that Maixner *et al.* [14] reported that nicotinically-induced contractions of the ileum were unaffected by clonidine. In the same study, NE inhibited the nicotinic contractions, as it had inhibited the DMPP-induced release of ACh from the longitudinal muscle preparation [12]. Why clonidine inhibited the DMPP-induced release of [^{3}H]ACh from our synaptosomal preparation but failed to affect the DMPP-induced contractions of the ileal segments is unclear. It may be, however, that the difference lies in the probability that the nicotinic contractions in the intact ileum are mediated through the direct action of DMPP on the ganglionic cell bodies while the DMPP-induced release of [^{3}H]ACh in our preparation is strictly a presynaptic phenomenon.

When the α_1-agonist phenylephrine was evaluated, it was determined that this drug was far less potent than either clonidine or NE as an inhibitor of the nicotinically-induced [^{3}H]ACh release from the synaptosomes. Similarly, it is less potent than clonidine as an inhibitor of the contractions

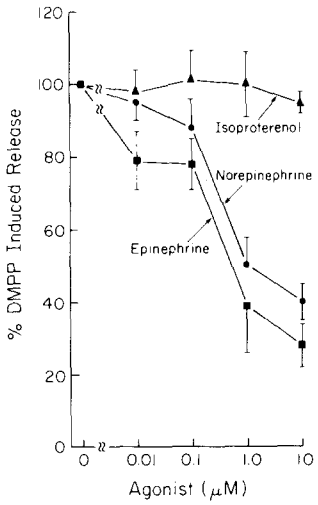

Fig. 1. Effects of α- and β-adrenergic agonists on the DMPP-induced release of [^{3}H]ACh. Key: (■) epinephrine, (●) norepinephrine, and (▲) isoproterenol. The tissue was incubated for 2 min at 37° in the presence of the drugs, and the reaction was stopped by returning the sample tubes to an ice bath. The basal release, which averaged approximately 12% of the total [^{3}H]ACh originally present in the tissue, was subtracted from the release in the presence of 10 μM DMPP to give the value of the DMPP-induced release. The data points are the mean ± S.E.M. from three separate experiments.

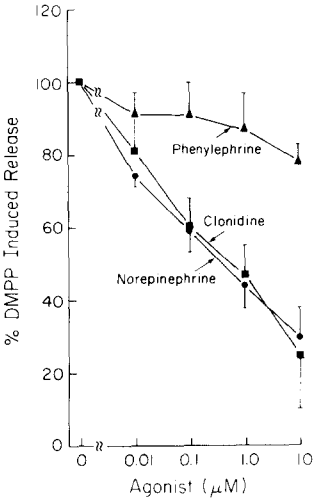

Fig. 2. Effects of α_1- and α_2-adrenergic agonists on the DMPP-induced release of [^{3}H]ACh. Key: (■) clonidine, (●) norepinephrine, and (▲) phenylephrine. The DMPP-induced release was as in Fig. 1. The data points are the mean ± S.E.M. from three separate experiments.

caused by electrical stimulation of the longitudinal muscle [6–8]. The relative potencies of clonidine and phenylephrine indicated that an α_2-receptor was involved in the inhibition of the DMPP-induced release of [^{3}H]ACh.

Effects of α-adrenergic antagonists on clonidine-induced inhibition of release. When the α_2-antagonist yohimbine was tested to determine if it could reverse the inhibition of release caused by clonidine, it was found that yohimbine itself caused inhibition of the basal [^{3}H]ACh release (Fig. 3). Kilbinger and Wessler [15] found no effect of yohimbine on the resting release of ACh from the longitudinal muscle-myenteric plexus preparation, while yohimbine increased the electrically-stimulated release of ACh from this tissue. It is interesting to note, however, that when Drew [7] and Andrejak *et al.* [8] were studying the ability of yohimbine to reverse clonidine's inhibition of cholinergically mediated contractions in the ileum, yohimbine caused reductions of the contractions itself. Perhaps an inhibition of the release of ACh may have been involved in this effect of yohimbine on the contractions. While the mechanism for the inhibition of the basal release of [^{3}H]ACh by yohimbine remains unexplained, one possible candidate could be the blockade of ionic channels by yohimbine which has been demonstrated previously [16, 17].

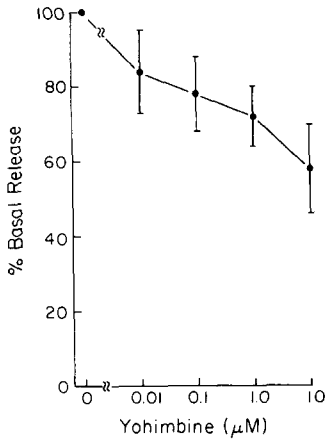

Fig. 3. Effects of increasing concentrations of yohimbine on the basal release of [^{3}H]ACh. The tissue was incubated for 10 min in an ice bath in the presence of yohimbine followed by a 2-min incubation at 37° to evaluate [^{3}H]ACh release. The values are the mean ± S.E.M. from three separate experiments.

To evaluate the effect of yohimbine on the action of clonidine in the synaptosomes, therefore, the inhibitory effect of yohimbine on [^{3}H]ACh release had to be overcome. To do this, all release (the leakage at 0°, basal, DMPP-induced and DMPP-induced plus clonidine) was evaluated in the presence of 1 μM yohimbine. Under these conditions, the absolute magnitudes of the basal and DMPP-induced releases were not affected and the effect of yohimbine on the action of clonidine could be determined. As can be seen in Fig. 4A, in the presence of yohimbine the dose–response curve of clonidine was shifted far to the right. The α_1-antagonist, prazosin, did not inhibit [^{3}H]ACh release on its own, nor did it reverse the inhibitory effects of clonidine (Fig. 4B).

In summary, E and NE inhibited the DMPP-induced release of [^{3}H]ACh from ileal synaptosomes, and isoproterenol was inactive. Clonidine reduced the release while phenylephrine was far less potent. Yohimbine reversed the inhibition of clonidine, but prazosin was ineffective. The data indicate that the inhibition by NE of the DMPP-induced release of [^{3}H]ACh from the synaptosomes of the

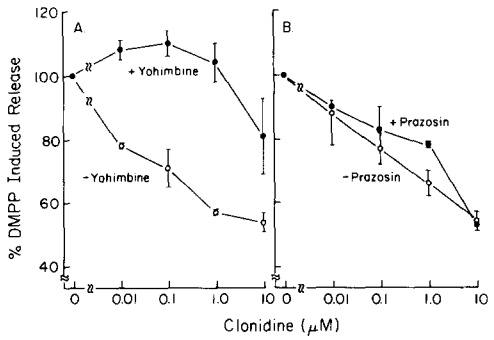

Fig. 4. Effects of 1 μM yohimbine (A) and 1 μM prazosin (B) on the inhibition by clonidine of the DMPP-induced release of [^{3}H]ACh. The tissue was incubated in the presence (●) and absence (○) of either yohimbine or prazosin for 10 min in an ice bath prior to the addition of clonidine. The values are the mean ± S.E.M. from three separate experiments.

myenteric plexus is mediated by an interaction with an α_2-adrenergic receptor located on the presynaptic nerve terminal. To date, it has been demonstrated that the synaptosomal preparation contains nicotinic, muscarinic, purinergic and noradrenergic receptors which are capable of modulating the release of [^{3}H]ACh. The preparation should be productive, therefore, in future investigations concerning the possible molecular mechanisms involved in that modulation.

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