DESENSITISATION AND AGONIST BINDING TO CHOLINERGIC RECEPTORS IN INTESTINAL SMOOTH MUSCLE

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1. Introduction

The development of affinity labels for the muscarinic acetylcholine receptor [1—4] has opened the way for studies on the binding of agonists (activators) with this receptor, using the inhibition of the binding of the affinity label as a measure of the binding of the agonist. There are, however, difficulties in this approach, since it is necessary to have the agonists present in concentrations far greater than those necessary to obtain a contractile response and also for extened period of time, conditions which lead to receptor desensitisation (see e.g. ref. [5]). In intact longitudinal muscle strips from the guinea-pig small intestine, as described here, and in microsomal fractions from homogenates of this tissue [6], the binding of agonists shows an apparent negative co-operativity (Hill coefficients approx. 0.4—0.5), while the binding curves for antagonists have, within experimental error, the slope predicted (Hill coefficient = 1), from a simple mass-action equilibrium. In this communication it is shown that the slope of the Hill plot for the binding of carbachol to intact muscle strips can be increased to approximately unity, with an increase in the concentration of carbachol required for half-maximal inhibition of the binding of the affinity label, tritiated propylbenzilylcholine mustard (^{3}H-PrBCM)*, if the tissue is exposed to a desensitizing dose of the agonist immediately prior to the labelling.

* *Abbreviation*: ^{3}H-PrBCM, *N*-2′-chloroethyl-*N*-[2″, 3″-$^3H_2$] propyl-2-aminoethyl benzilate.

2. Methods

Strips of the longitudinal muscle of guinea-pig small intestine [7] were suspended in Krebs—Henseleit solution gassed with 95% O_2—5% CO_2 at 30°C and preincubated for 1 hr. ^{3}H-PrBCM (final concentration of aziridinium ion 2.4×10^{-9} M) was then added and 4 or 10 min later the reaction terminated by transferring the strips to fresh Krebs and washing for a further 75 min with three further changes of the medium. Where appropriate receptor antagonists were added 30 min before, and agonists 30 sec or 1 min before the ^{3}H-PrBCM. The strips were blotted dry, weighed and bound tritium determined by liquid scintillation counting after solubilisation of the tissue. A more detailed description of the procedure has been given elsewhere [2].

The ^{3}H-PrBCM, specific activity 1.4 Ci/mmol or 1.8 Ci/mmol [2], was always converted into the aziridinium ion derivative, the pharmacologically active form [1], prior to addition to the medium by incubation in 10 mM phosphate buffer, pH 7.5, at room temperature for 1 hr [2].

Propylbenzilylcholine (*N*,*N*-dimethyl-*N*-propyl-2-aminoethyl benzilate) was kindly synthesised for us by Mr B. Peck, following the method of Ford—Moore and Ing [8].

3. Results and discussion

Approximately 85% of the binding of ^{3}H-PrBCM by longitudinal muscle strips could be inhibited by muscarinic agonists or antagonists. However, while antagonists yielded binding curves with a Hill slope not significantly

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Table 1
Slopes of Hill plots for the inhibition by muscarinic agonists and antagonists of the binding of ^{3}H-PrBCM

Antagonists		
Atropine	0.84 ± 0.06	(6)
Methylatropinium	0.95 ± 0.14	(11)
Propylbenzilylcholine	0.96 ± 0.14	(4)
Agonists		
Acetylcholine*	0.54 ± 0.06	(6)
Carbachol	0.44 ± 0.08	(9)
Hexyltrimethylammonium	0.51 ± 0.10	(9)
(+)-Acetyl-β-methacholine†	0.36 ± 0.06	(9)

The values are the slopes ± standard error, with the number of points in parenthesis, of plots of log [(100-% of uninhibited binding of ^{3}H-PrBCM) / (% of uninhibited binding − % non-specific binding)] *vs* Log [inhibitor]. Antagonists were added 30 min before, and agonists 1 min before the ^{3}H-PrBCM. Incubation with ^{3}H-PrBCM (2.4 nM) was for 10 min at 30°C. For all inhibitors the non-specific binding was taken as 15%.

* In the presence of 10^{-7} M neostigmine.
† In the presence of 10^{-6} M physostigmine.

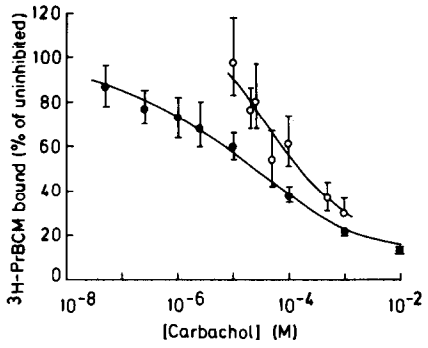

Fig. 1. Inhibition by carbachol or ^{3}H-PrBCM binding by muscle strips with (○) or without (•) prior desensitisation. Incubation with ^{3}H-PrBCM (2.4 nM) was for 4 min at 30°C. The points are values of the ratio 100 × ^{3}H-PrBCM bound in presence of inhibitor / ^{3}H-PrBCM bound in absence of inhibitor (15–22 strips for each point) with the approx. 69% confidence limits (vertical bars). For prior desensitisation (O) the strips were exposed to 10^{-4} M carbachol for 15 min then washed for 15 sec in Krebs–Henseleit solution containing the concentration of carbachol to be tested (zero for total binding) before transfer to Krebs–Henseleit solution containing ^{3}H-PrBCM with or without carbachol.

different from unity (table 1), as predicted by a simple mass-action equilibrium, all the agonists tested yielded curves with mean Hill slopes around 0.5, all being significantly different from unity. Similar observations have been made by Burgen and Hiley [6] for the binding of acetylcholine to a microsomal fraction derived from an homogenate of this tissue. The period of exposure to ^{3}H-PrBCM, which forms a covalent bond with the receptor, was kept as short as experimentally convenient since ultimately the binding of the irreversible label will overcome the inhibition by the reversible antagonist, giving too low a value for the inhibition (see ref. [9] for a discussion). However the rate of ^{3}H-PrBCM binding by the intact muscle strips is such that during the 10 min exposure employed in the experiments in table 1 only some 16% of the total number of receptors available are occupied [2] and under these conditions the reduction in the binding in the presence of an inhibitor should give a good measure of the true inhibition. Consistent with this reducing the period of exposure to ^{3}H-PrBCM form 10 to 4 min did not significantly after either the shape or the position of the percentage inhibition *vs* concentration curve for atropine. The shape of the inhibition curve for carbachol (fig. 1, filled points) was not significantly altered (mean Hill slope 0.40 ± 0.04) but the curve was shifted to the left. This suggested that the apparent negative co-operativity might be a consequence of receptor desensitisation, the rate of which is well known to be dependent on the concentration of agonist present and the time for which it acts, the implication being that desensitisation involves a change in receptor conformation with a decreased affinity for the agonist. Recovery from desensitisation, as measured by the recovery of the contactile response to an agonist is slow, although determination of the true rate of receptor recovery is complicated by the fact that in smooth muscle there is also a non-specific component to desensitisation [10]. Taking advantage of the slow recovery, muscle strips were exposed to 10^{-4} M carbachol for 15 min and then washed for 15 sec before labelling with ^{3}H-PrBCM for 4 min in the presence or absence of carbachol. The prior desensitisation led to only a small and statistically non-significant decrease in the binding of ^{3}H-PrBCM (12% and 12%, 2 experiments). However the inhibition curve for carbochol (fig. 1 open points) was both steeper (Hill coefficient 0.90 ± 0.21) and also shifted to the right. This suggests that, as with the nicotinic acetylcholine receptor in chick biventer muscle [11], the phenomenon of desensitisation of muscarinic acetyl-

choline receptors in the longitudinal muscle from guinea-pig small intestine is associated with a change in receptor conformation. It demonstrates in addition that this results in a decrease in the binding affinity for an agonist, carbachol (approx. 10^4 M^{-1} for the desensitised receptor). Further it seems probable that the Hill coefficients of approx. 0.4–0.5 observed for inhibition of ^{3}H-PrBCM binding *vs* concentration curves for agonists when there is no pretreatment with agonist reflects the increasing degree of desensitisation occurring during the course of the incubation as the concentration of agonist used as inhibitor is increased. This proposition is lent some support by the contrast with the inhibition by an agonist, decamethonium, of the binding of the α-toxin from *Naja nigricollis* by membrane fragments from the electroplax of *Electrophorus electricus* [12], a tissue which apparently does not show the phenomenon of desensitisation [13]. There the binding curve obtained for decamethonium approximated well to a rectangular hyperbola (Hill coefficient = 1) for most of its length and varying the time of preincubation with the agonist was without effect on the level of inhibition observed [12].

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