the activation of the eicosanoid system may involve specific receptors for LTC_4 in guinea pig lung. In fact pyrilamine, a histamine H_1-receptor antagonist, does not prevent TXA_2 generation in nonsensitized lungs due to LTC_4 (G. C. Folco, unpublished observation).

The remarkable potency of LTC_4 in triggering TXA_2 generation in sensitized lungs, might be partly due to concomitant release of histamine from sensitized cells caused directly by LTC_4. It is of interest in this context that lipoxygenase products have been shown to increase the release of histamine from perfused guinea pig lung [18]. Further studies are required to settle this point.

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On the nature of the interaction between chlorpromazine and the muscarinic receptor

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Chlorpromazine is an effective antagonist on a remarkable range of drug receptors and biochemical systems. At receptors for which it has a high affinity, such as histamine-H_1, α_1-adrenergic and dopaminergic, it seems very likely that it acts as a competitive inhibitor. However, on receptor systems for which chlorpromazine has a lower affinity it is not so certain that the interaction is competitive and there is seldom any experimental evidence which allows a firm conclusion to be drawn. Hill coefficients of unity for curves of the inhibition of the binding of ^{3}H-receptor ligands are consistent with either competitive or non-competitive inhibition. Similarly, a parallel shift of agonist log dose–response curves in intact tissues with an appreciable 'spare' receptor population could indicate either a competitive or an effectively irreversible blockade [1]. Chlorpromazine has potent membrane actions [2] and it is possible that with some receptors inhibition could be the result of a perturbation of the receptor–membrane interface, particularly at higher concentrations of the drug. There is some suggestive evidence that such could be the case with the muscarinic receptor.

Low concentrations of chlorpromazine produce a parallel shift of the log dose–response curve for the acetylcholine-induced contraction of the rabbit ileum, but at higher concentrations the curve is flattened [3]. This effect is similar to that of tetracaine [4], and for this local anaesthetic a study of the inhibition of the binding of [^{3}H]quinuclidinyl benzilate, [^{3}H]QNB, a selective ligand for the muscarinic receptor [5], indicated that at low concentrations the inhibition is competitive, but that at higher

concentrations the mechanism is more complex [4]. In addition, chlorpromazine seems to have long-lasting and complex effects on the binding of carbachol to muscarinic receptors in guinea-pig intestinal smooth muscle [6].

These observations have prompted us to investigate in more detail the interaction of chlorpromazine with the muscarinic receptor. To do this we have utilised the method adopted by Taylor, Wolf and Young [4], where the concentration of the drug required for 50 per cent inhibition of the receptor-specific binding of [^{3}H]QNB, the IC_{50}, is measured at various concentrations of [^{3}H]QNB. If the inhibition is non-competitive the IC_{50} is independent of the concentration of the ^{3}H-ligand. If the interaction is competitive then

$$IC_{50} = \frac{K_{QNB}}{K_{CPZ}} \cdot [^3\text{H-QNB}] + \frac{1}{K_{CPZ}},$$

where K_{CPZ} and K_{QNB} are the affinity constants of chlorpromazine and [^{3}H]QNB, respectively, for the muscarinic receptor. Thus a plot of IC_{50} against [^{3}H-QNB] should be linear.

Methods

Preparation of a crude synaptosomal fraction from rat caudate nucleus. Rats (Wistar strain, females, 35 days old, weighing 84 ± 3 g) were killed by decapitation and the heads immediately immersed in liquid nitrogen for 7 sec. Subsequent operations were carried out over ice in a cold-room. The brain was removed and the head of the caudate nucleus dissected out. The tissue was blotted and weighed and then homogenised in 10 vol. of cold 0.32 M sucrose using a teflon-glass homogeniser with a motor driven pestle (700 r.p.m., four strokes). The homogenate was then centrifuged at 250 *g* for 15 min, the pellet discarded and the supernatant recentrifuged at 10,000 *g* twice for 10 min. The resultant pellet was resuspended in 0.32 M sucrose–5 mM phosphate buffer, pH 7.4, divided into small portions in polypropylene tubes and stored frozen at −20° until required. A single preparation (from 25 animals) was used in all the experiments with chlorpromazine. Protein was determined by the method of Lowry *et al.* [7].

Binding measurements. Measurements were made in a modified Krebs-phosphate solution, pH 7.4, which contained (mM): NaCl 116, KCl 4.7, $MgSO_4$ 1.2, Na_2HPO_4 2.5 and $CaCl_2$ 1.25. (−)-[^{3}H]Quinuclidinyl benzilate ((−)-[^{3}H]QNB), specific activity 40.2 Ci/mmol was obtained from New England Nuclear. The concentration of [^{3}H]-QNB in diluted stock solutions was determined by scintillation counting of an aliquot. A typical incubation (total vol. 3.06 ml) contained 25 µl of diluted stock (−)-[^{3}H]QNB (final concentration 0.22 nM), 0.13 mg protein (i.e. 42 µg/ml) and the appropriate concentration of chlorpromazine. For other concentrations of (−)-[^{3}H]QNB the total volume of Krebs-phosphate solution was varied so that the relative *amounts* of the [^{3}H]-ligand and homogenate were kept constant. This was to maintain at a near constant level any depletion of the free (−)-[^{3}H]QNB concentration caused by protein binding. The maximum depletion which actually occurred was calculated to be *ca.* 8 per cent. Solutions of chlorpromazine hydrochloride (Sigma) in Krebs-phosphate solution were prepared fresh daily. Incubations at 30° were for 1 hr. At the end of this period a sample (1–12 ml, depending on the protein concentration) was filtered rapidly under vacuum through a Whatman GF/B glass fibre filter and the filter then washed 3 times with 5 ml ice-cold Krebs-phosphate solution. The filters were soaked in 10 ml Aquasol (New England Nuclear) overnight and the tritium then determined by liquid scintillation counting. Three to six measurements were made at each chlorpromazine concentration. The level of non-specific binding of (−)-[^{3}H]QNB was defined as the binding of (−)-[^{3}H]QNB insensitive to inhibition by 2 µM methylatropinium bromide (3 measurements at each (−)-[^{3}H]QNB concentration). The IC_{50} for chlorpromazine at each (−)-[^{3}H]QNB concentration, i.e. the concentration of chlorpromazine causing 50 per cent of the receptor-specific binding of (−)-[^{3}H]QNB, was determined from the inhibition curves obtained using 3–7 concentrations of chlorpromazine.

Inhibition of the contraction of guinea-pig intestinal smooth muscle. Measurements on the carbachol-induced contraction of a strip of the longitudinal muscle from guinea-pig intestine and the calculation of the affinity constant of chlorpromazine were made as described previously [4].

Results and discussion

The inhibition of the binding of (−)-[^{3}H]QNB to the rat striatal membrane fraction was measured at seven concentrations of (−)-[^{3}H]QNB ranging from 0.03 to 1.8 nM. At the lowest concentration the non-specific binding accounted for approximately 6 per cent of the total [^{3}H]QNB bound, but this increased to 15 per cent with 1.8 nM (−)-[^{3}H]QNB. At this concentration the inhibition curve for chlorpromazine was displaced to sufficiently high concentrations (IC_{50} 3 µM) that significant inhibition of the non-specific binding occurred. A similar observation has been made in studies of the inhibition of [^{3}H]QNB binding by local anaesthetics [4, 8]. To correct for this effect an inhibition curve was measured using 1.8 nM (−)-[^{3}H]QNB and with 2 µM methylatropinium bromide present in every incubation. Chlorpromazine, 4 µM, produced a 31 per cent inhibition of the methylatropine-insensitive binding of (−)-[^{3}H]QNB, which increased to 47 per cent with 10 µM inhibitor. The inhibition curves for chlorpromazine measured under standard conditions (no methylatropine present) were corrected for this inhibition of the non-specific binding, but only for the curves with the two highest concentrations of (−)-[^{3}H]QNB was the correction to the IC_{50} of any significance.

The variation of the corrected IC_{50} values for chlorpromazine with the concentration of (−)-[^{3}H]QNB present is shown in Fig. 1. The correlation coefficient, from simple linear regression, is 0.99, consistent with a competitive interaction between chlorpromazine and (−)-[^{3}H]QNB over the concentration range studied. The affinity constant for chlorpromazine derived from this graph was $1.8 \pm 3.3 \times 10^7\ M^{-1}$ and that for (−)-[^{3}H]QNB $3.5 \pm 6.4 \times 10^{10}\ M^{-1}$. The appreciable errors on both values

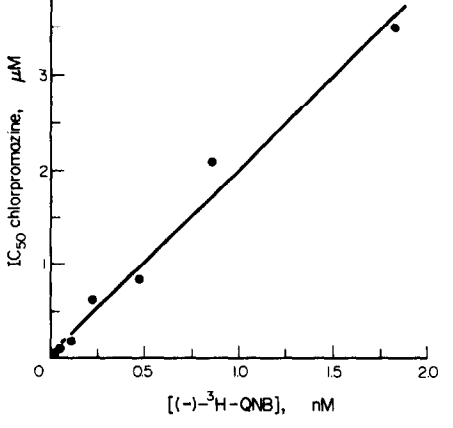

Fig. 1. Variation of the IC_{50} for chlorpromazine with the concentration of (−)-[^{3}H]QNB present. The line drawn is a best-fit line obtained from linear regression analysis.

indicate the limitations of this method for the determination of affinities. However, the value for (−)-[^{3}H]QNB is in good agreement with recent literature values for rat striatum, 2.3–5 × 10^{10} M^{-1} (see reference [4]). The value for chlorpromazine is in accord with the value obtained from inhibition of [^{3}H]QNB binding to rat brain, *ca.* 3 × 10^7 M^{-1} [9], but is higher than the values obtained from [^{3}H]propylbenzilylcholine mustard binding, 2.9 × 10^6 and 1.4 × 10^6 M^{-1} [6, 10] or from inhibition of the acetylcholine-induced contraction of the small intestine of the guinea-pig, 3.0 × 10^6 M^{-1} [11], rabbit, 3.5 × 10^6 M^{-1} [3] and rat, 8 × 10^5 M^{-1} [12]. The value determined from inhibition of the cyclic GMP response to carbachol in neuroblastoma cells was 5 × 10^5 M^{-1} [13].

In an attempt to confirm the value of affinity constant obtained from the binding study we have examined the action of chlorpromazine on carbachol-induced contractions of the guinea-pig ileum. However, the effect of increasing concentrations of chlorpromazine was complex (Fig. 2), in agreement with the study of Saxena and Johri [3]. There was an increasing depression of the maximum response, but the lower part of the curve appeared to be shifted in a near parallel fashion. The affinity constant estimated from this parallel shift was 1.7 ± 0.4 × 10^7 M^{-1}, in excellent agreement with the value of 1.8 × 10^7 M^{-1} derived from the binding experiments.

In summary, it appears that whereas low concentrations of chlorpromazine have distinct non-competitive effects on tissue response, there is no indication, in the concentration range studied, of a non-competitive action at the receptor level, as mirrored by the inhibition of the binding of (−)-[^{3}H]QNB.

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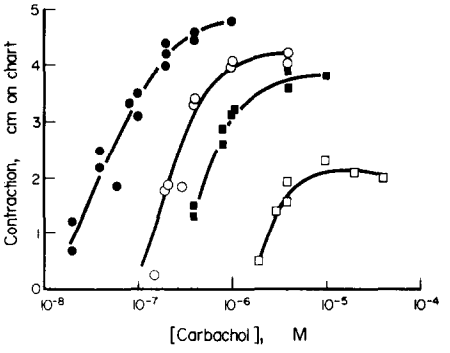

Fig. 2. Inhibition by chlorpromazine of the contractile-response to carbachol of the longitudinal muscle from guinea-pig small intestine. Concentration of chlorpromazine present (μM): ●, none; ○, 0.4; ■, 1 and □, 4. Chlorpromazine was present in the Krebs solution and was allowed to equilibrate with the tissue for 30 min after each change of concentration.

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