now been overcome by the introduction of the indicator Quin 2 which, as its acetoxymethyl ester, is membrane permeant (Tsien, 1981; Tsien et al., 1982). We have used Quin 2 to determine the resting level of $[\text{Ca}^{2+}]_{i}$ in rat brain synaptosomes and to examine the nature of the calcium transients due to depolarization by high potassium and glutamate levels. We have also used a cytochemical technique to localize sites of calcium sequestration in the synaptosome.

Synaptosomes were prepared from the forebrains of adult Wistar rats as described by Cotman & Matthews (1971) and resuspended at a protein concentration of 1 mg/ml in a Krebs–Ringer solution (145 mm NaCl, 5 mm KCl, 1.3 mm NaHCO$_3$, 1.2 mm NaH$_2$PO$_4$, 10 mm glucose, 2 mm CaCl$_2$, 20 mm Hepes, pH 7.5) equilibrated with 95% O$_2$/5% CO$_2$. The synaptosomes were incubated at 37°C for 30 min and then loaded with Quin 2 by incubation with 50 μM-quin 2 acetoxymethyl ester (from Dr. T. J. Rink, University of Cambridge). After incubation at 37°C for 30 min the synaptosomes were diluted tenfold with Krebs–Ringer solution, incubated for a further 30 min, washed twice by centrifugation and resuspended in Krebs–Ringer solution containing 1 mg/ml bovine serum albumin. The calibration of [Ca$^{2+}$], was carried out (Tsien et al., 1982) by determining fluorescence at 100% and 0% Ca saturation following synaptosomal lysis with 0.1% Triton X-100. Cytochemical localization of calcium was carried out using pyroantimonate (Burgoyne et al., 1983).

Following loading of synaptosomes the fluorescence emission spectrum corresponded to that of the hydrolysate product. The basal level of [Ca$^{2+}$], found was similar for several synaptosome preparations and corresponded to a mean level of 35 ± 6 nm (n = 7). Depolarization of synaptosomes with either 55 mM-K$^+$ or 200 μM-glutamate led to a transient increase in fluorescence signal (Fig. 1) indicating a rise in [Ca$^{2+}$],. The maximum value of [Ca$^{2+}$], attained was variable and lay in the range 145–660 nm for 55 mM-K$^+$ and 210–670 nm for 200 μM glutamate. However, the poor time resolution obtained was such that in most cases the rise in [Ca$^{2+}$], was not seen and only the decay was recorded. Therefore, the levels of [Ca$^{2+}$], reported in depolarized synaptosomes must be regarded as minimum values. The decay in the [Ca$^{2+}$], was rapid and resting levels of [Ca$^{2+}$], were reached within 1 min. This time course was similar to that for the calcium transient in the squid giant synapse (Miledi & Parker, 1981). It should be noted that 55 mM-K$^+$ and 200 μM-glutamate had no effect on the fluorescence of Quin 2 which, as its acetoxymethyl ester, is membrane permeant (Tsien et al., 1982). We have also used a cytochemical technique to localize sites of calcium sequestration in the synaptosome. The rapid decay of the [Ca$^{2+}$], transient in synaptosomes indicated that Ca$^{2+}$ is sequestered internally or removed by a plasma membrane transport mechanism within seconds of depolarization. Mitochondria (Nicholls & Akerman, 1981) and smooth endoplasmic reticulum (McGraw et al., 1980) have both been considered as sites of calcium sequestration in synaptosomes. Data from cytochemical localization of calcium binding sites in synaptosomes suggests that synaptic vesicles should be considered as alternative sites for calcium sequestration in the nerve terminal.

Fig. 1. Effect of depolarization on [Ca$^{2+}$], in synaptosomes.

Synaptosomes loaded with Quin 2 were depolarized by addition of 55 mM-K$^+$ or 200 μM-glutamate, as indicated. Fluorescence was recorded at 37°C at excitation 335 nm, emission 490 nm.

### Interaction of a nicotinic acetylcholine receptor from locust (Schistocerca gregaria) central nervous system with concanavalin A: comparison with vertebrate receptor

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We have previously reported the partial purification of an acetylcholine receptor with a predominantly nicotinic pharmacology from the supra-esophageal ganglion of the locust *Schistocerca gregaria* (Filibin et al., 1983). The specific binding activity of the purified protein was very variable and values ranging from 26 to 230 pmol of $^{125}$I-labelled α-bungarotoxin bound/mg of protein were obtained. The protein was purified by a combination of a detergent extraction and affinity chromatography on α-bungarotoxin coupled to Sepharose 4B. In the case of the nicotinic receptor from vertebrate tissues an additional affinity chromatography step involving lectins is often used and gives improved specific binding activities. Thus we investi-
gated the use of concanavalin A (Con A)-Sepharose 4B as an additional/alternative purification step.

Membrane fragments were prepared from the supraoesophageal ganglia of adult locusts (Schistocerca gregaria) exactly as described in Filbin et al. (1983). The membranes were solubilized in 10mM-potassium phosphate buffer (pH 7.4) containing 0.14M-NaCl, 1mMEDTA, 2mM-benzamidime hydrochloride, 0.1mM-benzethonium chloride, 0.1mM-PMSF, 1% (v/v) Triton X-100. Con A-Sepharose 4B beads (Shorr et al., 1981) (1ml) were washed with the above buffer (20ml) and the detergent-solubilized membrane (1ml) loaded and recycled through the column for 2 h at 4°C using a peristaltic pump (22ml/h). Non-bound material was then eluted with buffer (5ml). Specific elution of bound material was achieved by using α-methyl-D-mannoside in the sample buffer. Various concentrations of α-methyl-D-mannoside were used and in all cases eluted fractions (0.5ml) were retained and assayed for 125I-labelled α-bungarotoxin-binding activity (Filbin et al., 1983). We found that 0.1M-α-methyl-D-mannoside removed 110% of the applied α-bungarotoxin-binding activity from the column and elution with 1.0M-α-methyl-D-mannoside removed a further 15% of the binding activity, giving a total recovery of about 25% of the applied binding activity. We also noted that in most preparations about 10% of the α-bungarotoxin-binding activity of the detergent-solubilized membrane preparation failed to bind to the Con A column. These findings contrast with observations made by Anholt et al. (1980) who examined the behaviour of the receptor from electrophax tissue of Torpedo californica. They found that 0.2M-α-methyl-D-mannoside failed to elute any of the binding activity and application of 1.0M-α-methyl-D-mannoside resulted in the recovery of a maximum of 17% of the applied receptor activity. None of the receptor activity failed to bind to the Con A column.

Thus we concluded from these experiments that the locust receptor appeared to be heterogeneous with respect to its carbohydrate composition, as witnessed by the observation that 10% of the activity failed to bind to Con A-Sepharose. Additionally, the locust receptor that binds does so with a lower affinity than the electrophax receptor. With respect to the specific binding activity of the receptor eluted from the Con A column, we found that in many cases this was comparable with that observed by chromatography on α-bungarotoxin-Sepharose. Thus it appears that the receptor may constitute the bulk of the Con A-binding proteins in the locust solubilized-membrane preparations.

In the case of the receptor from Torpedo electrophax it is known that the binding of α-bungarotoxin is partially inhibited by Con A. Wonnacott et al. (1980) reported that up to 40% of the α-bungarotoxin binding sites on purified receptor from the electrophax of Torpedo marmorata could be blocked by Con A. These data were interpreted in terms of heterogeneity of the receptor molecules with respect to their carbohydrate moieties. We have now investigated this aspect of Con A binding in the locust. The binding of 125I-labelled α-bungarotoxin to a preparation of the locust receptor was measured in the presence and in the absence of various concentrations of Con A (0–50μg/ml). The binding of 125I-labelled α-bungarotoxin to a preparation of purified receptor from Torpedo electrophax was measured under the same conditions; the results are shown in Fig. 1. As previously observed, Wonnacott et al., 1980) we see inhibition of α-bungarotoxin binding to the receptor from Torpedo and this reaches a maximum of about 40%. In the case of the locust receptor, however, no inhibition of binding is seen even at the highest Con A concentration. Thus it appears that although Con A binds to the locust receptor, as witnessed by our experiments using Con A-Sepharose affinity chromatography, the disposition of binding sites on the receptor is quite different from that seen in Torpedo. We have additional evidence that in mammalian muscle the binding of Con A is primarily to the α-subunit of the receptor (G. Turnbull, unpublished work) and that receptor from Torpedo and from mammalian muscle are similar in that Con A binding is partially competitive with α-bungarotoxin binding.

Our findings lend further support to previous suggestions (Filbin et al., 1983) that although the locust receptor has a predominantly nicotinic pharmacology it may well have quite different physicochemical properties from its vertebrate counterpart.

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Fig. 1. Inhibition of the binding of 125I-labelled α-bungarotoxin to nicotinic cholinergic receptors from Torpedo marmorata electrophax and from supraoesophageal ganglia of Schistocerca gregaria

The binding of α-bungarotoxin was measured in the presence and absence of Con A. Results are means of triplicate experiments. , Torpedo receptor; , locust receptor.