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# γ-AMINOBUTYRIC ACID-STIMULATED CHLORIDE PERMEABILITY IN CRAYFISH MUSCLE

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## Summary

γ-Aminobutyric acid selectively increased $Cl^-$ permeability in isolated strips of crayfish abdominal muscle. Muscle fibers incubated in Van Harreveld's solution at room temperature took up $^{36}Cl^-$ to the extent of 700 ml/kg wet weight with a halftime of 2.5 min. During 15-s incubations, the control $^{36}Cl^-$ uptake space was 131 ± 4 ml/kg ($n = 60$) and this was significantly increased by γ-aminobutyric acid at 200 μM or higher concentrations to 177 ± 4 ml/kg ($n = 48$, $P < 0.05$). This effect was specific for chloride since γ-aminobutyric acid did not increase the uptake by crayfish muscle of radioactive sucrose, inositol, or propionate. γ-Aminobutyric acid stimulation of $^{36}Cl^-$ uptake is mediated by receptor-ionophore function since the process shows pharmacological properties virtually identical to those observed by electrophysiological techniques. The γ-aminobutyric acid stimulation of $Cl^-$ permeability is dose dependent with 50% of the maximal effect at 40 μM γ-aminobutyric acid and the dose vs. response curve is somewhat sigmoid. The γ-aminobutyric acid agonist muscimol causes the same maximal effect on $Cl^-$ uptake as γ-aminobutyric acid, but acts at 5-fold lower concentrations, i.e. is more potent. However, the partial agonist γ-amino, β-hydroxybutyric acid produced little or no stimulation of $^{36}Cl^-$ flux. The response to γ-aminobutyric acid was blocked by 2 mM β-guanidinopropionate or γ-guanidinobutyrate, 0.5 mM bicuculline, and 10 μM picrotoxinin. Picrotoxinin inhibition was dose dependent with 50% inhibition occurring at 4 μM. Antagonists did not affect control $^{36}Cl^-$ uptake. These results confirm electrophysiological observations that the postsynaptic response to the inhibitory neurotransmitter γ-aminobutyric acid involves a rapid increase in membrane permeability to $Cl^-$

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## Introduction

γ-Aminobutyric acid is a major neurotransmitter in the central nervous system of many animal phyla, and in the peripheral nervous system of, at least, the arthropod class of invertebrates. Thus γ-aminobutyric acid is selectively synthesized in certain (inhibitory) neurons, is released upon stimulation of such neurons, is transported into such neurons by a high affinity system which might serve to remove it from the synaptic cleft, and γ-aminobutyric acid mimics exactly the action of the inhibitory neurotransmitter on the target tissues [1—5].

The response of the target cells to γ-aminobutyric acid, limited to the immediate postsynaptic membrane surface, has been shown in several tissues by electrophysiological measurements to involve a rapid increase in membrane conductance to $Cl^-$ [3—11]. The molecular mechanism of this response has been approached by microelectrode studies on the quantity of γ-aminobutyric acid needed for a response, the ion specificity and extent of conductance increase, the kinetics of the response, and pharmacological aspects of the interaction of γ-aminobutyric acid, agonists, or antagonists with the postsynaptic receptor-ionophore sites.

The object of this report is to confirm with radioactive $Cl^-$ that γ-aminobutyric acid does increase the chloride permeability of the postsynaptic membrane, using the abdominal muscle of the crayfish. This demonstration provides a test-tube assay for mechanism and structure-function studies of γ-aminobutyric acid receptor-ionophore action.

## Experimental Procedures

*Materials.* $^{36}Cl^-$ (11.5 mCi/g Cl) and [$^{14}$C]sucrose (360 Ci/mol) were obtained from ICN. [2-$^{14}$C]Propionic acid, sodium salt (24 Ci/mol) was from Amersham Searle, and [$^3$H]myo-inositol (2.84 Ci/mmol) from New England Nuclear. γ-Aminobutyric acid, γ-amino, β-hydroxybutyric acid, β-guanidinopropionic acid, and γ-guanidinobutyric acid were obtained from Sigma. Bicuculline was purchased from Pierce Chemicals; bicuculline methiodide was synthesized from it as previously described [12] and converted to the methochloride salt. Picrotoxinin was purified from picrotoxin by the method of Jarboe and Porter [13]. Muscimol was a gift of Dr. P. Krogsgaard-Larsen.

*Methods.* Abdominal muscles were removed from crayfish (*Procambarus clarkii*) and placed in aerated Tris-buffered Van Harreveld's solution which had the following composition: NaCl (205 mM), KCl (5.4 mM), $CaCl_2$ (13.6 mM), $MgCl_2$ (2.6 mM), tris(hydroxymethyl)aminomethane (5 mM) and β-mercaptoethanol (2 mM), pH 7.7.

Small segments of the muscle were cut off and placed on a tissue slicer and gently pressed. Following this, muscle strips, each about 0.5—1 cm in length and $<0.1$ cm thick, were cut, tied with thread on one end, and allowed to equilibrate for 60 min in Van Harreveld's solution. All experiments were done at 22 ± 3° C.

*Total $^{36}Cl^-$ uptake.* Following equilibrium, muscle strips were transferred to vials containing 5 ml of Van Harreveld's solution containing $^{36}Cl^-$ (0.1 μCi/ml,

0.4 μCi/mmol). The muscle strips (5—8 per experimental condition) were withdrawn from the tracer solution at various time intervals, blotted gently on a filter paper and weighed on a Sartorius® electrobalance. The muscle strips were then solubilized overnight at room temperature with 0.2 ml of Soluene (Packard). To the solubilized material was added 5 ml of a scintillation fluid containing 3.0 g of 2,5-diphenyloxazole per l of toluene and counted in a Beckman CPM-100 counter. Also counted were tissue blanks and 100 μl of the $^{36}Cl^-$-containing incubating medium. $^{36}Cl^-$ space was determined as follows:

$$\frac{\text{cpm/kg wet weight tissue}}{\text{cpm/ml incubation medium}} = \text{ml/kg wet weight tissue}$$

Since the counting efficiency was the same for all the samples, this ratio is the same whether expressed as cpm or dpm. The size of the chloride space was remarkably reproducible although occasionally it varied from the average. This is probably due to occasional inclusion of different types of muscle cell in the experiment.

To study the effect of γ-aminobutyric acid on $^{36}Cl^-$ uptake, equilibrated muscle strips were transferred to vials containing a given concentration of γ-aminobutyric acid in 5 ml of Van Harreveld's solution containing $^{36}Cl^-$ as above, and the uptake studied for exactly 15 s. The muscle strips were then removed, blotted, weighed, solubilized and prepared for counting as described earlier. Typically muscle strips of approx. 15 mg would take up 480 ± 50 cpm during 15 s without γ-aminobutyric acid and 675 ± 60 cpm with $2 \cdot 10^{-4}$ M γ-aminobutyric acid.

To study the effect of various antagonists on γ-aminobutyric acid-induced $^{36}Cl^-$ uptake, muscle strips were incubated with the antagonist for 5 min prior to their transfer to $^{36}Cl^-$ medium which also contained the same concentration of the antagonist in addition to the agonist.

## Results

### *$^{36}Cl^-$ uptake*

$^{36}Cl^-$ uptake was studied as a function of time. After equilibration for 60 min in Van Harreveld's solution, muscle strips were exposed to $^{36}Cl^-$-containing medium for various times. Fig. 1 illustrates the total $^{36}Cl^-$ uptake by the crayfish tail muscle strips. Uptake of $^{36}Cl^-$ was at least biphasic, with an initial rapid phase during the first 5 min, followed by a slower phase with an apparent equilibrium being reached in 30 min. Thus the $^{36}Cl^-$ space resulting at 30 min (699 ± 27 ml/kg wet weight tissue, $n = 4$) was the same ($P < 0.05$) as the $^{36}Cl^-$ space at 60 min (660 ± 37 ml/kg wet weight tissue, $n = 4$).

To further characterize the $^{36}Cl^-$ uptake, the muscle strips were exposed to an extracellular space marker ([$^{14}$C]sucrose, 0.15 μCi/ml of incubation medium). The muscle strips did not take up very much [$^{14}$C]sucrose with time, and an apparent equilibrium with extracellular space was reached in 30 min. The [$^{14}$C]sucrose space at 30 or 60 min was 62 ± 4 ml/kg wet weight ($n = 6$). This small extracellular space was confirmed with radioactive [$^3$H]inositol and the anionic [$^{14}$C]propionate. Furthermore, γ-aminobutyric acid did not significantly alter the sucrose space. Thus the sucrose space in presence of a supramaximal

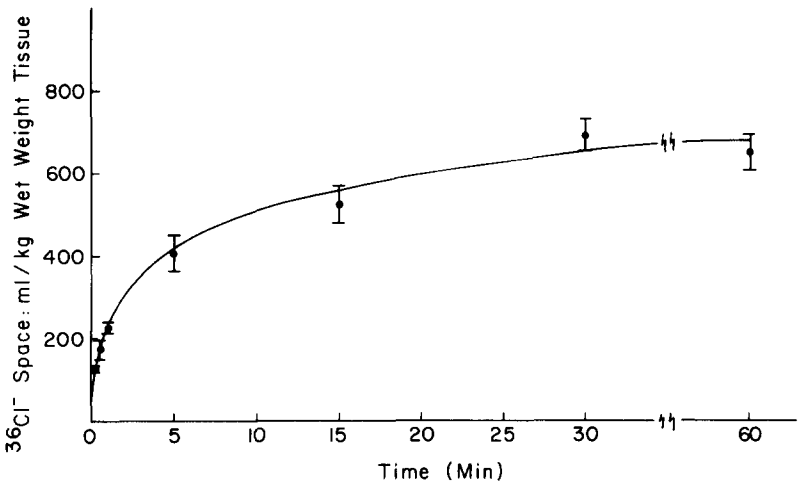


Fig. 1. Total $^{36}Cl^-$ uptake by crayfish muscle strips. Tissues were incubated for the designated periods of time in $^{36}Cl^-$ (0.1 μCi/ml) Van Harreveld's solution. Each point represents the mean ± S.E. of 4—8 observations.

concentration of γ-aminobutyric acid ($10^{-3}$ M) was 64 ± 5 ml/kg wet weight ($n$ = 6). Since the total $^{36}Cl^-$ uptake in terms of $Cl^-$ space is far more than the [$^{14}$C]-sucrose space, it is apparent that much of the $^{36}Cl^-$ represents intracellular uptake. However, several pools including extracellular space are likely to be included. As with sucrose, no effect of γ-aminobutyric acid was observed on the uptake of [$^3$H]inositol or [$^{14}$C]propionate.

*Effect of γ-aminobutyric acid and γ-aminobutyric acid agonists on $^{36}Cl^-$ uptake*

Since γ-aminobutyric acid is an inhibitory neurotransmitter in crayfish and other invertebrates and is believed to cause an increase in $Cl^-$ permeability of the postsynaptic muscle cell membrane [4,5], it was of interest to study the effects of γ-aminobutyric acid on $^{36}Cl^-$ uptake in crayfish tail muscle. A time of exposure (15 s) was chosen in which background $Cl^-$ uptake would be small but which would still allow a γ-aminobutyric acid stimulation to be seen if one should occur. Strips of abdominal muscle tissue were exposed to $^{36}Cl^-$ medium with or without a concentration of the agonist ($2 \cdot 10^{-4}$ M γ-aminobutyric acid) that produces maximal conductance change in crayfish [9—11,14—16]. The presence of γ-aminobutyric acid in the uptake medium for 15 s caused a significant increase in $^{36}Cl^-$ uptake (Table I). γ-Aminobutyric acid-induced $^{36}Cl^-$ uptake, expressed as $^{36}Cl^-$ space (177 ± 5 ml/kg wet weight tissue, $n$ = 48) was significantly greater ($P < 0.05$) than the control $^{36}Cl^-$ uptake (131 ± 4 ml/kg wet weight tissue, $n$ = 60). Thus a 15 s exposure of the muscle strips to $2 \cdot 10^{-4}$ M γ-aminobutyric acid produced an increase in the $^{36}Cl^-$ space of about 46 ml/kg. Supramaximal concentrations of γ-aminobutyric acid also produced an increase in the $^{36}Cl^-$ space of the same magnitude as that of a maximal concentration: thus $1 \cdot 10^{-3}$ M γ-aminobutyric acid increased $^{36}Cl^-$ space to 175 ± 8 ml/kg ($n$ = 19, Table I). No significant drop in response was observed at supramaximal concentrations. Furthermore, preincubation of the tissue with γ-aminobutyric acid ($10^{-4}$—$2 \cdot 10^{-3}$ M) for 5 min prior to assaying $^{36}Cl^-$ uptake

TABLE I

EFFECTS OF AGONISTS AND ANTAGONISTS ON $^{36}Cl^-$ UPTAKE

Tissues were equilibrated in the physiological medium for 60 min at room temperature and then transferred for exactly 15 s to vials containing $^{36}Cl^-$ (0.1 $\mu$Ci/ml) with or without the agonist. To study the effect of antagonists, the tissues were pre-equilibrated with the antagonist for 5 min and the same concentration of the antagonist was present during the 15 s uptake. The $^{36}Cl^-$ uptake is presented as the $^{36}Cl^-$ space (mean ± S.E.) with the number of tissues in parentheses.

| Agent | Concentration (M) | $^{36}Cl^-$ uptake ($^{36}Cl$ space, ml/kg wet wt. tissue) (mean ± S.E.) |
|---|---|---|
| None (control) | | 131 ± 4 ($n$ = 60) |
| γ-Aminobutyric acid | $2 \cdot 10^{-4}$ | 177 ± 4 ($n$ = 48) |
| | $5 \cdot 10^{-4}$ | 179 ± 9 ($n$ = 10) |
| | $1 \cdot 10^{-3}$ | 175 ± 8 ($n$ = 19) |
| Muscimol | $5 \cdot 10^{-5}$ | 174 ± 8 ($n$ = 16) |
| γ-Amino, β-hydroxybutyric acid | $2 \cdot 10^{-3}$ | 143 ± 10 ($n$ = 16) |
| γ-Aminobutyric acid | $2 \cdot 10^{-4}$ | |
| + β-guanidinopropionic acid | $2 \cdot 10^{-3}$ | 134 ± 10 ($n$ = 10) |
| + γ-guanidinobutyric acid | $2 \cdot 10^{-3}$ | 150 ± 8 ($n$ = 13) |
| + bicuculline | $5 \cdot 10^{-4}$ | 137 ± 13 ($n$ = 7) |
| + picrotoxinin | $1 \cdot 10^{-5}$ | 139 ± 8 ($n$ = 6) |

with $2 \cdot 10^{-4}$ M γ-aminobutyric acid did not cause any greater, nor lesser, increase in $^{36}Cl^-$ uptake. This indicates that the increase in $^{36}Cl^-$ uptake caused by γ-aminobutyric acid during the first 15 s exposure to $^{36}Cl^-$ is the maximum obtainable. Also there does not appear to be desensitization of the γ-aminobutyric acid receptors.

γ-Aminobutyric acid produced a concentration-dependent increase in the $^{36}Cl^-$ uptake by crayfish abdominal muscle (Fig. 2). Under constant conditions,

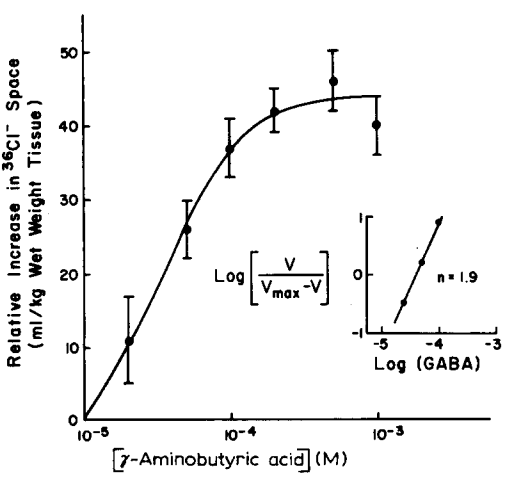


Fig. 2. Concentration-dependent effect of γ-aminobutyric on $^{36}Cl^-$ uptake. Tissues were incubated with various γ-aminobutyric acid concentrations in $^{36}Cl^-$ Van Harreveld's solution for 15 s. Each point represents the mean ± S.E. of 5—8 observations.

the $Cl^-$ space increment due to 15 s exposure to γ-aminobutyric acid is taken as the response. This dose vs. response curve for γ-aminobutyric acid-stimulated $^{36}Cl^-$ flux is virtually identical to the dose vs. response curve as obtained for conductance increase in crayfish leg muscle as induced by γ-aminobutyric acid [5,9,14,16,17]. A Hill plot (insert in Fig. 2) of this limited data on $Cl^-$ flux had a slope of 1.9 ± 0.5, indicating some cooperativity, just as was found in the conductance studies. Since the plot included only three useable points with standard errors of 10%, the slope value is not very accurate, but clearly greater than one.

Muscimol has been shown to potently mimic the action of γ-aminobutyric acid on vertebrate central neurons [18,19], crustacean stretch receptor [20] and muscles [21] and insect muscle (Miller, T. and Olsen, R.W., unpublished). This agonist, at a concentration of $5 \cdot 10^{-5}$ M, caused the same maximal increase in $^{36}Cl^-$ uptake by crayfish muscle (174 ± 8 ml/kg, $n = 16$) as $2 \cdot 10^{-4}$ M γ-aminobutyric acid (Table I). Thus muscimol is a full agonist more potent than γ-aminobutyric acid itself.

*Effect of a partial agonist on $^{36}Cl^-$ uptake*

γ-Amino,β-hydroxybutyric acid resembles γ-aminobutyric acid in that it also increases chloride conductance at crustacean inhibitory neuromuscular junctions. However, it does not produce the same maximal response as γ-aminobutyric acid: thus γ-amino,β-hydroxybutyric acid, at maximal concentrations, $\geqslant 2 \cdot 10^{-3}$ M [10], produces only about 70% of the conductance change relative to γ-aminobutyric acid and can be classified as a partial agonist for this tissue [10,11]. The effect of several concentrations up to $2 \cdot 10^{-3}$ M γ-amino,β-hydroxybutyric acid for 15 s on $^{36}Cl^-$ uptake was determined. The maximal effect obtained is shown in Table I. This compound produced only a small statistically insignificant increase in $^{36}Cl^-$ uptake (131 ± 4 to 143 ± 10 ml/kg, $n = 16$). This response is not nearly as great as that to full agonists γ-aminobutyric acid and muscimol, and is consistent with γ-amino,β-hydroxybutyric acid acting as a partial agonist.

*Effect of antagonists on γ-aminobutyric acid-induced $^{36}Cl^-$ flux*

The effect of various pharmacological agents on the γ-aminobutyric acid-induced $^{36}Cl^-$ uptake was studied. Muscle strips were exposed to the antagonists for 5 min prior to their transfer to vials containing $^{36}Cl^-$ with or without agonist and antagonist for 15 s. Table I shows that γ-aminobutyric acid-induced $^{36}Cl^-$ uptake was sensitive to competitive γ-aminobutyric acid receptor antagonists [5,10,11,16] like β-guanidinopropionic acid ($2 \cdot 10^{-3}$ M) and γ-guanidinobutyric acid ($2 \cdot 10^{-3}$ M). The γ-aminobutyric acid-induced $^{36}Cl^-$ uptake was also inhibited by $5 \cdot 10^{-4}$ M bicuculline and partially by $3 \cdot 10^{-4}$ M bicuculline methochloride. Furthermore, $10^{-5}$ M picrotoxinin inhibited almost completely (84 ± 10%, $n = 6$) the γ-aminobutyric acid-induced $^{36}Cl^-$ uptake (Table I, Fig. 3). The same concentration of picrotoxinin had no effect on control $^{36}Cl^-$ uptake. Picrotoxinin caused a concentration-dependent inhibition of γ-aminobutyric acid-induced $^{36}Cl^-$ uptake as shown in Fig. 3. These concentrations of picrotoxinin also correspond with those active at inhibiting the inhibitory neuromuscular junctions in arthropods [5,14,17].

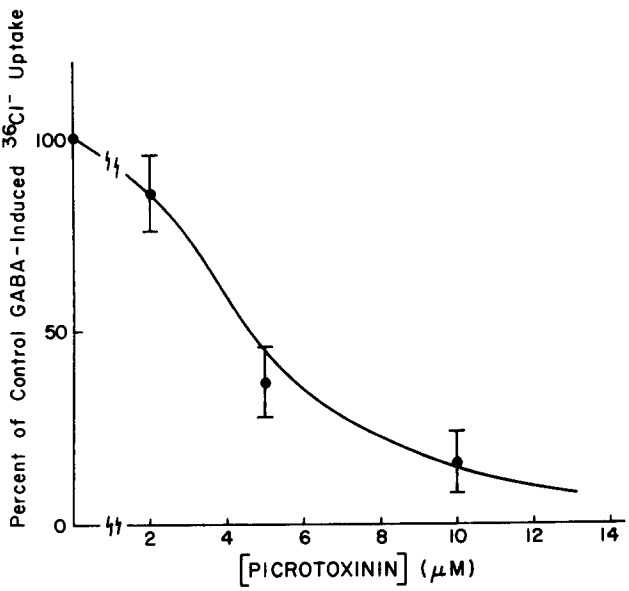


Fig. 3. The inhibitory effect of picrotoxinin on γ-aminobutyric acid-induced $^{36}Cl^-$ flux. Each point represents mean ± S.E. of 4—6 observations.

## Discussion

The physiological response to the neurotransmitter γ-aminobutyric acid, which acts on arthropod muscle cells [4,5] and neurons of various organisms [1—4], is a decrease in resistance of the postsynaptic membrane. The response is dependent upon the external $Cl^-$ concentration and appears to involve an increased passive conductance to that anion [5—11]. Because the chloride equilibrium potential is close to that of the resting potential, this response is inhibitory since it prevents subsequent excitation. The membrane macromolecule(s) responsible for controlling the chloride flux, the ionophore, appears to be regulated by a regulatory protein subunit (or subunits), the receptor, situated on the outer surface of the postsynaptic membrane. Binding of the neurotransmitter molecule to the receptor is associated with one or more active states of the ionophore, while the unliganded receptor is associated with an inactive state of the ionophore. The invertebrate neuromuscular synapses, both excitatory and inhibitory, appear to function in a rapid manner analogous to that of the vertebrate neuromuscular junction. However, the neurotransmitter for vertebrate skeletal muscle is acetylcholine (nicotinic) while the invertebrate muscle transmitters are γ-aminobutyric acid (inhibitory) and, probably, glutamate (excitatory) [4].

Binding of ligand appears to provide the necessary energy for conformational interconversions of the different states of these receptor-ionophores. The membrane conductance responses occur with a time-course of milliseconds [4—7, 22—25] and apparently without covalent bond changes.

While electrophysiological techniques have provided much information about receptor function, test-tube assays for the response to neurotransmitters

can be complementary in analyzing the molecular events. Of particular interest are the receptor phenomena of cooperativity in dose vs. response curves, partial agonism, and desensitization, as well as the physicochemical properties of the macromolecules involved in the physiological function of regulating membrane ion permeability. Furthermore, a rapid assay for screening potential γ-aminobutyric acid synapse-blocking drugs and for analyzing the chemical requirements for such action would be useful.

The ionic permeability changes induced by neurotransmitters in a variety of target tissues as detected by electrophysiological techniques have also been studied with radioactive ions. Thus cholinergic agonists increase $Ca^{2+}$, $K^+$, $Na^+$, or $Cl^-$ permeability in isolated strips of smooth or striated muscle (refs. 26 and 27, reviewed in ref. 28), and $Na^+$ permeability in glands [29], cultured nerve cells [30] and neuroblastoma [31] and in cell-free homogenates of electroplax tissue enriched in acetylcholine receptor-ionophores [32]. Likewise, catecholamines increase the flux of $K^+$ in smooth muscle [33,34].

This report extends such effects to the inhibitory transmitter γ-aminobutyric acid, which induces a specific increase in $^{36}Cl^-$ uptake during brief exposures of crayfish tail muscle fibers. While the crayfish muscle has a small extracellular space, indicated by radioactive sucrose, inositol, and propionate, several chloride compartments are likely to exist, as found in barnacle muscle [35]. The tissue used in these studies showed fairly rapid equilibration with chloride but still allowed detection of some stimulated uptake by the neurotransmitter, which is known to act rapidly. The compartment affected by γ-aminobutyric acid is at least 46 ml/kg, which is not increased by longer exposure to γ-aminobutyric acid. The fact that the increase caused by γ-aminobutyric acid is saturable with respect to concentration of the neurotransmitter argues that a finite compartment of the tissue is responsive to γ-aminobutyric acid and that the response is rapid. Thus a dose vs. response curve appears to be a valid treatment of the data.

This increase in $^{36}Cl^-$ uptake caused by γ-aminobutyric acid is shown to be mediated by γ-aminobutyric acid receptor-ionophores since it is specific for $Cl^-$, mimicked by agents such as muscimol which are active on γ-aminobutyric acid receptors in neurophysiological studies, and inhibited by compounds which act as antagonists of γ-aminobutyric acid synaptic action, such as γ-guanidinobutyrate, β-guanidinopropionate, bicuculline, and picrotoxinin. Furthermore, the dose vs. response curves for the response to γ-aminobutyric acid and its inhibition by picrotoxinin are remarkably similar to those determined by conductance measurements on crustacean muscle. This assay therefore provides a means for quantitative structure-function studies of agents active on the γ-aminobutyric acid receptor and/or ionophore. In vitro binding studies have been correlated with receptor action [36—43], but a test-tube assay of γ-aminobutyric acid ionophore activity has not been described previous to this. It is hoped that this approach will also be applicable to cell-free homogenates of tissue enriched in membrane-bound γ-aminobutyric acid binding proteins in crayfish muscle [37] and rat brain [39].

The chloride permeability increase, as measured here, showed a dose dependency on γ-aminobutyric acid concentration with 50% of the maximal effect observed at 40 μM. Very similar concentration dependence was seen for con-

ductance responses to bath-applied γ-aminobutyric acid in arthropod tissues [5, 11,14—17,44]. In those cases a markedly sigmoid curve was uniformly observed, with slopes of 2—4 for Hill plots of the dose vs. response curves. The relatively small effect seen here, compared to the large background $Cl^-$ flux, makes it difficult to determine whether there is any sigmoidicity in the dose vs. response curve, but the data expressed in a Hill plot does give a straight line with a slope of 1.9, indicating some cooperativity. The mechanism whereby this cooperativity arises is not known, however, it could be at the level of γ-aminobutyric acid receptor binding or at the level of ionophore activation [5,40]. As in electrophysiological studies on crayfish muscle [4,5], * no desensitization was observed in γ-aminobutyric acid-stimulated chloride flux following preincubation with γ-aminobutyrate.

Interestingly, the γ-aminobutyric acid agonist muscimol causes the same maximal effect on $Cl^-$ uptake as γ-aminobutyric acid, but at roughly 5-fold lower concentration, as occurs also for γ-aminobutyric acid-like activity of muscimol on various preparations (refs. 18—21, Miller, T. and Olsen, R.W., unpublished). Muscimol and its analogues potently block γ-aminobutyric acid binding to tissue sites having the chemical specificity of receptors for γ-aminobutyric acid [39]. The potency and specificity of muscimol for γ-aminobutyric acid receptors make it a potentially useful tool for in vitro studies of γ-aminobutyric acid receptors and also in treatment of nervous disorders related to γ-aminobutyric acid deficiencies.

On the other hand, γ-amino,β-hydroxybutyric acid is a partial agonist on crustacean tissue [10,11], but maximal concentrations induce only a small, if any, increase in $^{36}Cl^-$ uptake under the conditions used here. This discrepancy does not seem to be due to diffusion barriers and is unexplained at this time. A similar effect was observed in vertebrate smooth muscle, where high doses of muscarinic partial agonists did not significantly mimic full agonists, even in part, in increasing $^{45}Ca^{2+}$ flux [26]. In the case of electroplax membrane homogenates, partial agonism of $^{22}Na$ flux was observed for nicotinic acetylcholine analogues, matching the electrophysiological data [32]. These observations support the principal of partial agonism, since the responses to such agents are clearly different from those of full agonists.

Partial agonism can be explained by the existence of conformation transition states of the receptor-ionophores. Most models propose two states (one active (ignoring "desensitization"), refs. 22—25). However, it is also possible that more than one active ionophore conformational state might exist (perhaps as many as a spectrum of them). In such a case, different ligands could cause different responses (efficacy) by activating ionophores of different permeability properties. Some evidence from crayfish muscle suggests that at least two active conformational states for γ-aminobutyric acid receptor-ionophores are a possibility (three-state model). Thus, changes in the ion specificity of the γ-aminobutyric acid ionophore were found with low vs. high doses of bath-applied γ-aminobutyric acid [5,11].

More evidence supports the two-state model in which partial agonism is theoretically due to non-exclusive binding to the active state [24]. In this case, non-productive binding to the inactive state of the receptor-ionophore would

* See note added in proof p. 528.

lead to fewer activated neurotransmitter-receptor complexes per unit time than for full agonists. This is equivalent to the model in which the ligand-bound receptor-ionophore complex of a partial agonist "flips" into and out of the active form less frequently than that of a full agonist [23].

The potency of inhibitors for the γ-aminobutyric acid-stimulated $^{36}Cl^-$ uptake also agreed well with electrophysiological assays. Thus inhibition occurred with 2 mM β-guanidinopropionate or γ-guanidinobutyrate, 0.5 mM bicuculline, 0.3 mM bicuculline methochloride, and 10 μM picotoxinin. As observed with conductance experiments [3—5,14—16], picrotroxinin is a more potent inhibitor than bicuculline of γ-aminobutyric acid responses in crayfish muscle. γ-Aminobutyric acid receptors in vertebrate [1,3,45] as well as invertebrate [3,4,12,14,46] neurons seem to be more sensitive to bicuculline than those of muscle, and β-guanidinopropionate acts as an agonist on neuronal γ-aminobutyric acid receptors but only as an antagonist on muscle γ-aminobutyric acid receptors [5,10,11,16]. However, since several drugs have similar effects on the two types of receptors, the differences may be a function of intracellular or extracellular milieu, experimental procedure, etc. Picrotoxin inhibition of γ-aminobutyric acid synapses has been reported to be non-competitive with γ-aminobutyric acid [5,17], and the drug does not inhibit γ-aminobutyric acid binding proteins [36—43]. Thus it appears to inhibit the chloride ionophore, and since most chloride ionophores in many tissues are coupled to γ-aminobutyric receptors, picotoxinin should prove a useful tool in analyzing γ-aminobutyric acid postsynaptic events. On the other hand, bicuculline is a fairly poor antagonist. Its effects may still be interesting, since the inhibition of γ-aminobutyric acid synapses also appears non-competitive with γ-aminobutyric acid [5,14], while it does inhibit γ-aminobutyric acid binding proteins [36—43]. At the effective doses of bicuculline, however, the effects of this drug are not specific for γ-aminobutyric acid receptor-ionophores [12,40]. Effects of other drugs on this postsynaptic response to γ-aminobutyric acid are presented elsewhere [47].

In conclusion, this assay provides a means of measuring interesting aspects of γ-aminobutyric acid receptor-ionophore structure and function.

These results have appeared in abstract form [48].

NOTE ADDED IN PROOF (received 26th November, 1976)

Desensitization of γ-aminobutyric acid receptors has been reported in some, but not all, crustacean muscles [49]. The fibers studied in this report (abdominal superficial flexor muscles [50], resemble the crayfish leg opener muscles in that they do not exhibit desensitization, and β-guanidinopropionate has antagonist action.

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## References


1 Curtis, D.R. and Johnston, G.A.R. (1973) Ergeb. Physiol. Biol. Chem. Exp. Pharmakol. 69, 97—188
2 Iversen, L.L. (1973) Perspectives in Neuropharmacology (Snyder, S.H., ed.), pp. 75—111, Oxford University Press
3 Krnjević, K. (1974) Physiol. Rev. 54, 418—540
4 Gerschenfeld, H.M. (1973) Physiol. Rev. 53, 1—129
5 Takeuchi, A. (1976) GABA in Nervous System Function (Roberts, E., Chase, T.N. and Tower, D.B., eds.), pp. 255—267, Raven Press, New York
6 Boistel, J. and Fatt, P. (1958) J. Physiol. 144, 175—191
7 Ozawa, S. and Tsuda, K. (1973) J. Neurophysiol. 36, 805—816
8 Takeuchi, A. and Takeuchi, N. (1966) J. Physiol. 183, 433—449
9 Takeuchi, A. and Takeuchi, N. (1967) J. Physiol. 191, 575—590
10 Dudel, J. and Kuffler, S.W. (1961) J. Physiol. 155, 543—562
11 Takeuchi, A. and Takeuchi, N. (1975) Neuropharmacology 14, 627—641
12 Olsen, R.W., Ban, M. and Miller, T. (1976) Brain Res. 102, 283—299
13 Jarboe, C.H. and Porter, L.A. (1965) J. Chromatogr. 19, 427—433
14 Shank, R.P., Pong, S.F., Freeman, A.R. and Graham, L.T. (1974) Brain Res. 72, 71—78
15 Swagel, M.W., Ikeda, K. and Roberts, E. (1973) Nat. New Biol. 244, 180—181
16 Feltz, A. (1971) J. Physiol. 216, 391—401
17 Takeuchi, A. and Takeuchi, N. (1969) J. Physiol. 205, 377—391
18 Johnston, G.A.R., Curtis, D.E., DeGroat, W.D. and Duggan, A.W. (1968) Biochem. Pharmacol. 17, 2488—2489
19 Naik, S.R., Guidotti, A. and Costa, E. (1976) Neuropharmacology 15, 479—484
20 Hori, N., Ikeda, K. and Roberts, E. Submitted
21 Wheal, H.V. and Kerkut, G.A. (1976) Brain Res. 109, 179—183
22 DelCastillo, J. and Katz, B. (1957) Proc. R. Soc. Lond. Ser. B, 146, 362—381
23 Katz, B. and Miledi, R. (1973) J. Physiol. 230, 707—717
24 Changeux, J.-P., Benedetti, L., Bourgeois, J.-P., Brisson, A., Cartaud, J., Devaux, P., Grunhagen, H., Moreau, M., Popot, J.-L., Sobel, A. and Weber, M. (1975) Cold Spring Harbor Symp. Quant. Biol. XL, 211—229
25 Sheridan, R.E. and Lester, H.A. (1975) Proc. Natl. Acad. Sci. U.S. 72, 3496—3500
26 Ticku, M. and Triggle, D.J. (1976) Gen. Pharmacol. 7, 133—140
27 Jenkinson, D.H. and Nicholls, J.G. (1961) J. Physiol. Lond. 159, 111—127
28 Bolton, T.B. (1973) Drug Receptors, (Crang, H.P., ed.), Macmillan, pp. 87—104, London
29 Kondo, S. and Schulz, I. (1976) Biochim. Biophys. Acta 419, 76—92
30 Catterall, W.A. (1975) J. Biol. Chem. 250, 1776—1781
31 Stallcup, W.B. and Cohn, M. (1976) Exp. Cell Res. 98, 277—284
32 Kasai, M. and Changeux, J.-P. (1971) J. Membrane Biol. 6, 1—80
33 Wahlstrom, B.A. (1973) Acta Physiol. Scand. 89, 522—530
34 Jenkinson, D.H. and Morton, I.K.M. (1967) J. Physiol. Lond. 188, 373—386
35 Gayton, D.C. and Hinke, J.A.M. (1971) Can. J. Physiol. Pharmacol. 49, 323—330
36 Olsen, R.W., Lee, J.M. and Ban, M. (1975) Mol. Pharmacol. 11, 566—577
37 Meiners, B., Kehoe, P., Shaner, D.M., Braunstein, S. and Olsen, R.W. (1977) Fed. Proc., in the press
38 Olsen, R.W. and Greenlee, D. (1976) Fed. Proc. 35, 1647, Abstract. 1480
39 Greenlee, D., VanNess, P. and Olsen, R.W. (1977) submitted
40 Olsen, R.W. (1976) GABA in Nervous System Function (Roberts, E., Chase, T.N. and Tower, D.B., eds.), pp. 287—304, Raven Press, New York
41 Young, A.B., Enna, S.J., Zukin, S.R. and Snyder, S.H. (1976) GABA in Nervous System Function (Roberts, E., Chase, T.N. and Tower, D.B., eds.), pp. 305—317, Raven Press, New York
42 Peck, E.J., Schaeffer, J.M. and Clark, J.H. (1976) GABA in Nervous System Function (Roberts, E., Chase, T.N. and Tower, D.B., eds.), pp. 319—336, Raven Press, New York
43 DeFeudis, F.V., Balfagon, G., Rosa de Sagarra, M., Madtes, P., Somaza, E. and Gervas-Camacho, J. (1975) Exp. Neurol. 49, 497—505
44 Brookes, N. and Werman, R. (1974) Mol. Pharmacol. 9, 571—579
45 Bowery, N.G. and Brown, D.A. (1974) Br. J. Pharmacol. 50, 205—218
46 McLennan, H. (1973) Can. J. Physiol. Pharmacol. 51, 774—775
47 Olsen, R.W. and Ticku, M.K. (1977) Brain Res., submitted
48 Ticku, M.K. and Olsen, R.W. (1976) Annual Meeting Society for Neuroscience Abstracts, Vol. II, p. 803
49 Dudel, J. and Hatl, H. (1976) Pflügers Arch. 364, 217—222
50 Evoy, W.H. and Bernek, R. (1972) Comp. Gen. Pharmac. 3, 178—186