

ANALYSIS OF MAUTHNER CELL
RESPONSES TO IONTOPHORETICALLY DELIVERED
PULSES OF GABA, GLYCINE AND L-GLUTAMATE

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SUMMARY

1. The intracellularly recorded responses of goldfish Mauthner neurones to iontophoretically applied pulses of amino acids have been analysed: their time courses have been compared with each other, and with those predicted from diffusion theory.

2. The rise time of the response to GABA is slower than that to glycine or L-glutamate. The response curves of the latter substances were very similar, and unlike that of GABA were markedly affected by increasing the distance of pipette-tip from the membrane. The results suggest that the time course of the responses to glycine and L-glutamate are determined mainly by free diffusion in the brain tissue (at least within about 200 μm of the cell), while that to GABA must be rate-limited by other factors, e.g. drug-receptor activation time.

3. The possibility that the responses are influenced by some desensitizing process was investigated by applying a second (test) drug pulse during the response to a prior conditioning one. In the case of glycine and of L-glutamate there was no attenuation of the response to a second pulse at any time. With GABA, however, the second response was reduced during the period of the conditioning response; the reduction was progressively less marked the later the test pulse occurred. A similar effect with GABA was seen when glycine was used as the test pulse. The responses to long-maintained drug pulses also indicated that for GABA, but not for glycine or glutamate, there seems to be some desensitizing process present.

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4. Calculated time courses of responses to brief pulses of glycine and of L-glutamate (based upon diffusion theory) differed somewhat from the observed curves, largely during the falling phase. However, when the calculations were based upon second-order reactions (two molecules of drug per receptor) the diffusion model gave results very like the observed ones.

5. Possible implications of these results for the role these three amino acids may have as neuro-transmitters are mentioned.

INTRODUCTION

The investigation of neuro-transmitters or putative transmitters, and of the actions of substances which block their effects, has been considerably extended by the introduction of the iontophoretic technique of drug application. From an analysis of the time courses of responses to brief pulses of a substance delivered at some fixed distance from a cell, some of the kinetics of drug-receptor activation can be deduced. The now classic studies of this type are those of Katz and his collaborators on the neuromuscular junction (del Castillo & Katz, 1955, 1957*a, b, c*; Katz & Thesleff, 1957*a, b*). These workers found that many depolarizing agents (acetylcholine (ACh), carbachol, nicotine, succinylcholine) caused rapid responses whose time courses depended chiefly upon the rate of diffusion of the drug to the receptor sites on the outside of the membrane. However, anticholinesterases caused a pronounced change in the time course of the ACh response, especially its duration, suggesting that the disappearance of drug molecules from the receptor region is normally not determined by simple diffusion. The ACh antagonist, curare, has a much slower action than that predicted by diffusion rate-limitation, as if there was a slow dissociation of a curare-receptor complex.

Little comparable information is known for the receptor sites on cells in the vertebrate central nervous system, and clearly neurones embedded in a matrix of neural and non-neural elements are not ideal cells on which to make such analyses. However, because of its ease of location, and the possibility of recording its responses to brief iontophoretic pulses reasonably accurately, the Mauthner cell seemed an appropriate one on which to make an attempt. The experiments described below were therefore designed to allow a comparison on the Mauthner cell of the responses to GABA, glycine, and L-glutamate. The results bear on the questions whether or not the time course of the responses is mainly determined by diffusion (i.e. access-limited), whether some feature of the drug-receptor interaction could be a limiting step, and whether the duration of the responses is shortened by some inactivating process.

METHODS

The techniques of recording from Mauthner cells and applying substances to them iontophoretically are as described by Diamond (1968) and in the accompanying paper (Diamond, Roper & Yasargil, 1973). Whenever the time courses of two or three different substances were compared, double- and triple-barrelled micro-pipettes were prepared and filled with the appropriate solutions (GABA 4M, pH 4.5; glycine 2M, pH 2.8-3.0; L-glutamate 2M, pH 7.0); in this way the positions of the points of release for the drugs could differ by at most the sum of the individual diameters of the drug pipette tips, which totalled 2-4 μm . In the experiments in which the effects of two consecutive pulses of the same drug were being tested, triple-barrelled micro-pipettes were used, the tips of which were level and in a line (i.e. in cross-section, 000); the outer two barrels were filled with the drug, while the

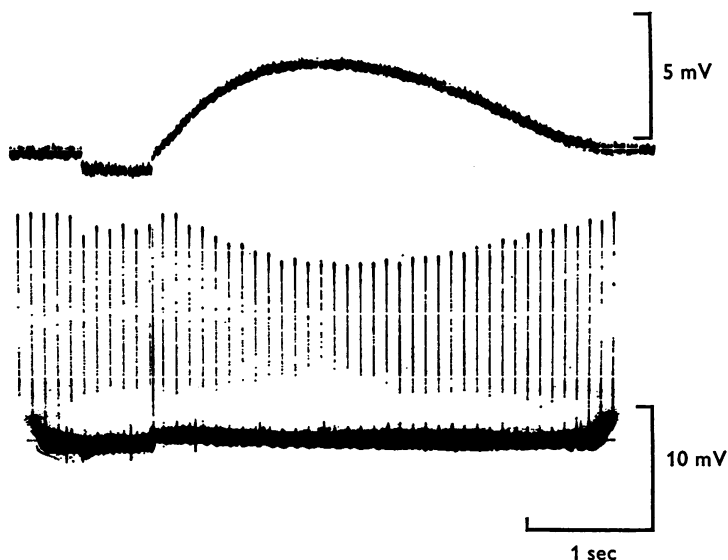


Fig. 1. Conductance and membrane potential change produced by iontophoretically applied L-glutamate. Upper trace, membrane potential at high gain, d.c. coupled. Lower trace, simultaneously recorded low-gain record of a train of antidromic impulses, a.c. coupled. A 500 msec pulse of L-glutamate (2×10^{-8} A) was applied at the beginning of the record (bar below bottom trace). The reduction of spike amplitude measures the decrease in input resistance (increase in membrane conductance). Note, the depolarization and increased membrane conductance follow nearly the same time course.

intervening barrel was left filled with distilled water; the outer two barrels were approximately 2 μm apart. By this arrangement the effect of iontophoretic ejections of the drug from one barrel upon the subsequent ejection from the alternate barrel were minimized (see del Castillo & Katz, 1957a).

The responses to the glycine and GABA applications gave measures of the *conductance change* of the membrane (see Diamond, 1968). This is a more direct measure of the drug-receptor activation than e.g. membrane potential changes, which are

functions of both the conductance change and the driving potential for the ionic current, as well as the time constant of the membrane. When a *brief* pulse of glutamate was applied, the conductance increase of the membrane had a time course which approximated to that of the concomitant depolarization and therefore we have used this as an adequate measure of the drug-receptor interaction (Fig. 1).

For a comparison of the time courses of responses to drug pulses it was essential to use approximately equal durations of current for ejection. When one drug is much less potent or less soluble in aqueous solution than another, significantly longer iontophoretic pulses may be required to pass enough of the first substance to produce comparable effects. In such a case, time courses of responses cannot readily be compared; a lengthy application (say 1–10 sec) could saturate nearby receptors, while affecting a much greater extent of receptor field than is the case for the shorter pulse (say 1–100 msec) (see Waud, 1967*a, b*). In the present studies, GABA, glycine, and L-glutamate pulses were kept at comparable durations so that such problems did not arise.

Temperature changes were effected by altering the temperature of the perfusion fluid passing through the mouth and over the gills of the fish. The temperature of the fluid before and after entering the experimental chamber was monitored, and a small thermistor placed in contact with the fish's brain just rostral to the cerebellum recorded its temperature (within about $\pm 1^\circ\text{C}$ accuracy). Small bits of dry ice placed on the chamber near the skull helped to keep the brain temperature down near the influx temperature when the lower ranges (*ca.* 6°C .) were studied.

RESULTS

The GABA response. The time courses of the responses to brief pulses of glycine and L-glutamate differed markedly from those to GABA (Fig. 2). Depending on the dose, and the distance of the pipette tips from the cell, the effects of glycine and glutamate reached a maximum in 0.1–1.0 sec and declined relatively slowly over the next 0.5–4.0 sec. However, the response to a similar pulse of GABA, applied from the same pipette assembly (i.e. from the same point relative to the neurone), reached its peak much later (e.g. 1–3 sec). The slow rise of the GABA response cannot be explained solely on the grounds of diffusion; the diffusion coefficients in aqueous solution of the three amino acids are unlikely to differ by more than a factor of about 1.2 (see Longsworth, 1953; Herz, Zieglansberger & Farber, 1969). It seems therefore that in the case of GABA, it was the activation of the receptors, or some subsequent step, which was rate-limiting, rather than the rate at which drug molecules arrived at the cell.

Distance changes. If the effects of L-glutamate and glycine on the Mauthner cell were access-limited by diffusion, and those of GABA limited by a relatively slow receptor-activation rate, then withdrawal of the drug pipette away from the membrane should affect the former (diffusion-limited) responses to a greater degree than it would the latter (reaction-rate-limited) ones (see del Castillo & Katz, 1957*a*). This was found to be the case for glycine and L-glutamate, considered with respect to GABA. That is, as the iontophoretic pipette was withdrawn, the time courses of

the responses to the more rapid-acting drugs (glycine and L-glutamate) began to approach those of the slower time course of GABA, in both rate of rise and in duration (Fig. 3). Practical measures of the shape of the responses are T_{\max} and $T_{\frac{1}{2}}$ where T_{\max} is the time to peak response and $T_{\frac{1}{2}}$ is the time to that when the response has fallen to one half its peak value, both measured from the onset of the current pulse. Table 1 gives the

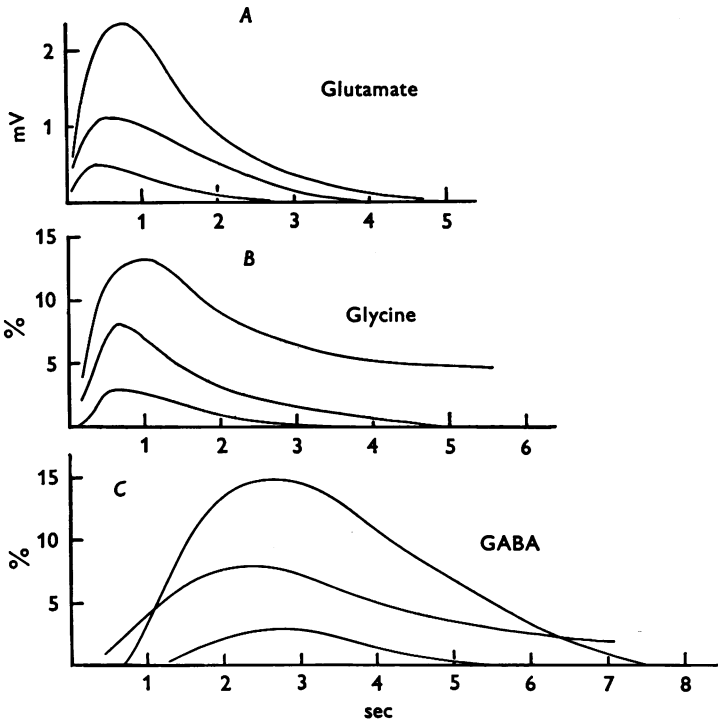


Fig. 2. Time courses of the responses to increasing doses of L-glutamate (top), glycine (middle) and GABA (bottom), applied iontophoretically to the Mauthner cell from adjacent barrels of a triple-barrelled micropipette in one experiment. Ordinates, response (mV depolarization for glutamate, % reduction of antidromic spike amplitude for glycine and GABA). Abscissae, time in seconds from the moment when the iontophoretic pulses were applied. Note, the time courses of responses to L-glutamate and glycine are similar, whereas that to GABA is much slower in onset. *A*: 50, 100, 200 msec pulses of L-glutamate (1.2×10^{-7} A). *B*: 50, 100, 200 msec pulses of glycine (1.6×10^{-7} A). *C*: 50, 100, 200 msec pulses of GABA (1.5×10^{-7} A).

results for three glycine/GABA experiments, showing that the time course of glycine responses were nearly always more sensitive to this displacement than were those of GABA (this is especially apparent in experiment no. 3 of this Table). Similar results were found when glutamate responses were

TABLE 1. The effect of distance upon the time course of responses to iontophoretically applied drugs (see text). A double-barrelled (glycine/GABA) micropipette was positioned near the Mauthner cell and several doses of each amino acid given. Then the pipette was withdrawn vertically about 30–100 μm and the doses repeated at this far position. The time to maximum response (T_{max}) and the time to $\frac{1}{2}$ decay of the response ($T_{\frac{1}{2}}$) was measured at both near and far positions. Three different experiments are shown

Experiment	Drug	Ratio, T_{max} (far/near)	Ratio, $T_{\frac{1}{2}}$ (far/near)
1	Glycine	1.4	1.3
	GABA	1.7	1.1
2	Glycine	2.5	3.8
	GABA	1.6	2.1
3	Glycine	7.5	7.1
	GABA	2.4	1.6

Note: T_{max} and $T_{\frac{1}{2}}$ of the glycine responses are increased to a greater extent by the displacement of the iontophoretic pipette than are T_{max} and $T_{\frac{1}{2}}$ of the GABA responses, especially in the third experiment shown.

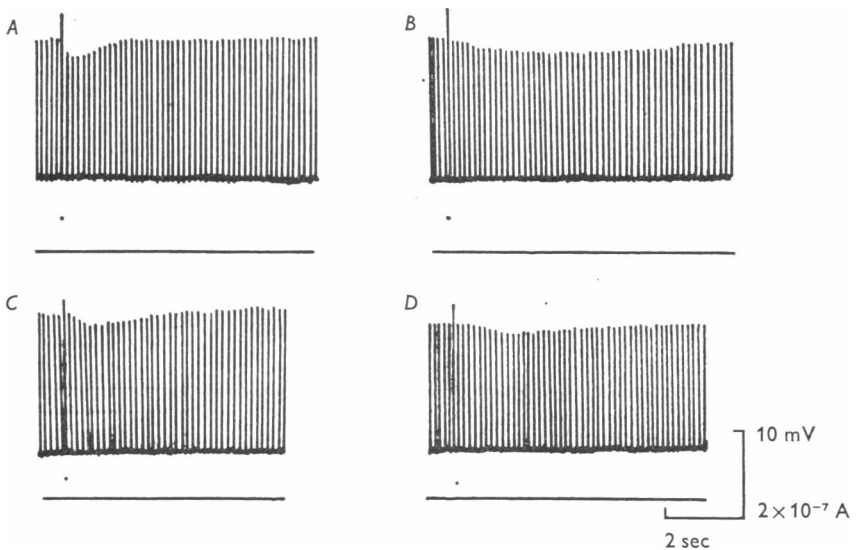


Fig. 3. The response of the Mauthner cell to glycine (upper traces) and GABA (lower traces) when the iontophoretic pipette was near the cell (*A*, *B*) and when it was withdrawn 30 μm vertically from the initial position (*C*, *D*). Glycine and GABA were applied from adjacent barrels of a double-barrelled micropipette. *A*, *B*, *C* and *D* show the intracellularly recorded antidromic spikes (upper traces) and the current passed through the iontophoretic pipette (upwards deflexion in lower traces). *A*, *C* the responses to glycine, *B*, *D* the responses to GABA.

compared to GABA responses. Our results, although less striking, resemble the findings of del Castillo & Katz (1957*a*) at the neuromuscular junction, where the rapid responses to carbachol were compared with the slow

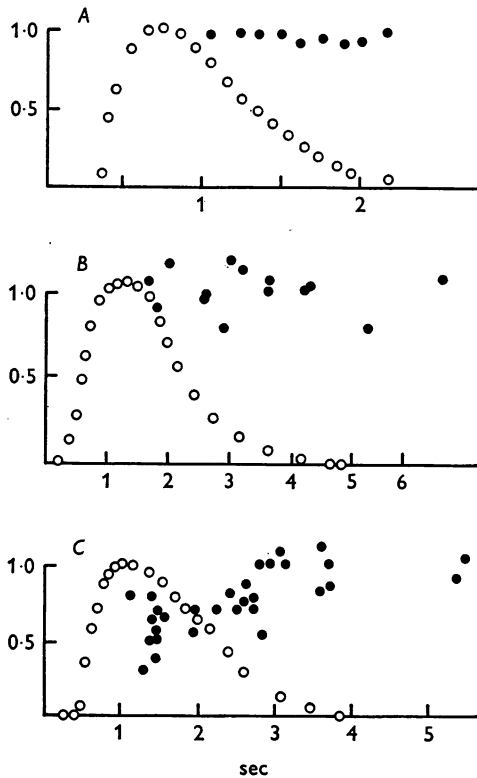


Fig. 4. Effect of an initial conditioning dose of amino acid upon a subsequent test dose of the same amino acid. Current pulses were passed through the two outer barrels of a 'linear' triple-barrelled pipette (see text), which were filled with the amino acid. Open circles in each graph are complete responses to a single conditioning dose, and the filled circles show only the maximum value attained by successive test doses. Each filled circle refers to the value of an individual test response which was superimposed upon the conditioning response, and which reached its peak at that point. Ordinate, responses measured as a reduction of an antidromic spike (for glycine and GABA) and depolarization (for glutamate). For ease of comparison, the responses have all been normalized taking the test responses as 1.0. *A* shows the responses of L-glutamate (test response was 4 mV), *B* of glycine (test was 15% spike reduction), *C* of GABA (test was 2-5% spike reduction). *A* and *B* are individual experiments, while *C* represents data pooled from three GABA experiments. Note: there is no effect of an initial dose of either L-glutamate or glycine upon the subsequent doses of these amino acids. There is a depression of GABA sensitivity, however, following the initial GABA dose.

curare responses for various positions of the curare-carbachol pipette tip from the motor end-plate. The present findings are consistent with the view that in these experiments the time courses of glycine and L-glutamate responses are probably determined by free diffusion through the brain tissue (at least that within 100–200 μm of the Mauthner cell), whereas the response to GABA may not be access-limited, but is being slowed by drug-receptor interaction or some subsequent step.

Desensitization. To test whether the decline of the response to a single application of a drug represents the fall in concentration at the receptors due to simple diffusion, or whether it is also affected by some rapidly acting 'desensitizing' process, the following experiment was done: two pulses of the same drug, of equal duration, were applied to the Mauthner cell alternately, from the two outer barrels of a 'linear' triple pipette (see Methods). The intervals between the pulses were chosen so that second 'testing' pulses occurred at various moments after the peak of the first 'conditioning' response. Provided that the responses were within the linear portion of the dose-response curve, the results showed that for L-glutamate and glycine there is no attenuation of a second response during any time after the peak response to the first pulse; the conditioning and test response sum algebraically (Fig. 4*a, b*).

However, the same experiment carried out with GABA showed a marked reduction of a test response when it occurred during the decline of a prior conditioning response (Fig. 4*c*). As the interval between the conditioning and testing pulses of GABA was increased, the response to the test pulse gradually recovered. In these experiments small doses of the drugs were used both for conditioning and testing pulses to avoid saturation of the receptors; all the responses, including the 'summed' conditioning and test responses (i.e. the response to a *single* dose equal to the sum of the conditioning and test doses) remained on the approximately linear regions of the dose-response curves.

In related studies, long-duration (2 min or longer) iontophoretic applications of glycine and L-glutamate gave responses which showed no signs of 'desensitization' (Fig. 5*a, b*); the membrane potential change in response to a long L-glutamate pulse, and the membrane conductance increase caused by a steady glycine efflux, remained constant after reaching its peak, provided that excessive iontophoretic currents were avoided. However, the results obtained with GABA seemed different; in the most extreme example (Fig. 5*c*) in which a very long steady pulse of GABA was applied, the response reached a maximum within 15 sec and then declined until at about 45 sec it reached a steady value which was maintained for more than 3 min. Since this type of result never occurred during maintained glycine or L-glutamate applications, it seems unlikely to be an

artifact caused by a change in the rate of GABA ejection during the constant current pulse.

GABA-glycine interaction. In some preliminary experiments, it was found that the reduction of the response to a second (test) pulse applied soon after the peak response to an initial (conditioning) dose of GABA, also held true when glycine was tested

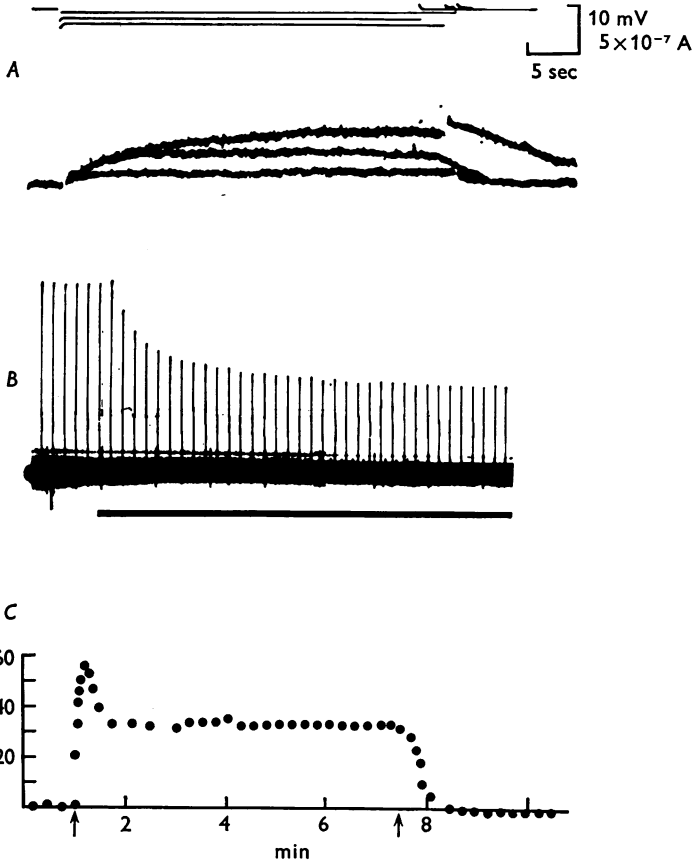


Fig. 5. Response of Mauthner neuron to prolonged iontophoretic application of L-glutamate, glycine and GABA. *A*: depolarizations (lower traces) caused by passing current through the L-glutamate pipette (downwards deflections in upper traces). *B*: intracellularly recorded antidromic spikes (upper trace) and the application of 100 nA through the glycine micro-pipette (during the time indicated by solid bar below trace). The decrease in spike amplitude measures the resultant conductance change. *C*: inhibitory conductance change (% decrease in antidromic spike amplitude) during long application of GABA (5×10^{-7} A, between times indicated by arrows). Ordinate in *C*, % decrease of spike height. Note: there is no decrease in the response to maintained doses of glutamate and glycine, but there is some indication of an 'inactivation' process during the prolonged GABA application. Voltage and time calibrations refer to *A* and *B*.

after a GABA dose. However, the converse was not found; that is, glycine had no equivalent effect upon subsequent GABA doses. Although in these earlier experiments the two drug-filled barrels were not separated by an intervening one, it is improbable that coupling between the barrels accounted for the result, since corresponding effects upon the release of GABA after glycine would then have been expected. GABA followed by glycine gave results like GABA followed by GABA, which were clearly different from those of the reverse sequence, glycine followed by GABA.

Temperature effects. The effects of temperature changes on the responses to the amino acids were tested to determine how far the time courses were sensitive to temperature, and whether this might implicate the existence of some chemical (e.g. enzymic) rather than physical (i.e. diffusion) limiting step.

In theory, the change of temperature *per se* should not affect the shape of the time course (T_{\max} , $T_{\frac{1}{2}}$) of the diffusion of the drug to the receptors, even though it will of course alter the absolute rate of diffusion and thus the rates of rise and fall. The great difficulty in these experiments is that changing the temperature of the brain is very likely to cause a relative displacement of the iontophoretic pipette tip, and this must have contributed a major source of the variability seen in our results.

Varying the brain temperature from 6 to 27° C had little consistent effect upon the shape of the time course of responses of iontophoretically applied drugs. Results from five particularly stable recordings are shown in Table 2. It appears that neither the rising nor the falling phase of the L-glutamate, glycine or GABA responses are preferentially affected by temperature alterations when measured in this fashion.

The diffusion model. Attempts were made to determine if the rapid time course of the responses to glycine or L-glutamate could be described in terms of simple diffusion of the substances to the cell membrane (since the response to GABA does not appear to be access-limited, it cannot be treated as a diffusional problem in the same way). Following del Castillo & Katz (1955) in their model of iontophoretic application at the motor end-plate, it is assumed that the drug is released instantaneously from a point source in an infinitely large homogeneous medium, that the receptors are situated at a point, that the response is proportional to the concentration of drug at that point, and that saturation of local receptor sites does not occur. Then

$$[\text{DRUG}]_t = \frac{Q}{8 (\pi Kt)^{1.5}} \exp(-r^2/4Kt),$$

where

$[\text{DRUG}]$ = drug concn. at receptors, at time t ,

Q = quantity of drug released at time $t = 0$,

r = distance of pipette tip from receptors,

K = diffusion coefficient.

It is particularly advantageous in the present instance to use a modification of this equation, *which makes it unnecessary to know the values of r , Q and K* , which are all constant, and which are all extremely difficult, if not impossible, to ascertain exactly in the experimental situation.

$$[\text{DRUG}]_t = [\text{DRUG}]_{\text{max}} \times \frac{C_o}{T^{1.5}} \exp(-\frac{3}{2}T),$$

where $[\text{DRUG}]_t$ = concn. (i.e. the response) at membrane at time t .

$[\text{DRUG}]_{\text{max}}$ = max. concn. reached at membrane (i.e. the maximum response at time t_{max}).

$$C_o = \frac{Q}{8r^3 (\frac{1}{3}\pi)^{1.5}}; \text{ this is constant for a fixed distance, } r, \text{ and fixed dose, } Q$$

T = the dimensionless variable t/t_{max} .

Fig. 6 shows plots of observed time courses of responses to glycine and L-glutamate, along with the corresponding theoretically predicted concentration rise and fall ($[\text{DRUG}]_t$), scaled to the same axes. Each

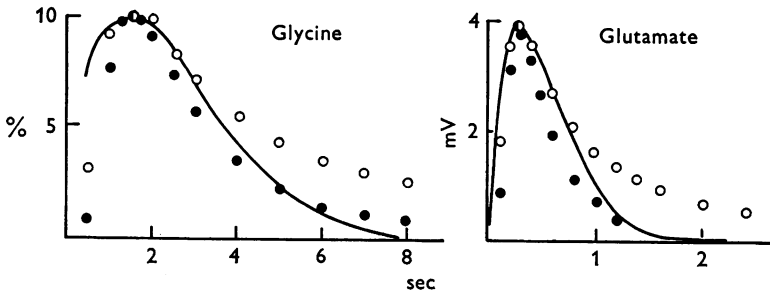


Fig. 6. The time courses of responses to iontophoretically applied glycine and L-glutamate compared with the time courses of the theoretically predicted change in drug concentration at the cell membrane. Brief pulses of glycine (left) and L-glutamate (right) were given at zero time on abscissae. Continuous lines show the actual responses to single applications of the drug, and the circles show the concentrations at the receptor site, calculated on the basis of free diffusion (see text for assumptions and derivation). Open circles, calculations based on a first-order drug-receptor interaction. Filled circles, based on a second-order interaction. Ordinates: for glutamate, mV depolarization, and for glycine, inhibitory conductance change (% reduction of antidromic spike amplitude). Abscissae: time in seconds from moment of drug application. Note: in both cases the observed responses decline more rapidly than predicted on the basis of first-order concentration changes (open circles).

theoretical curve is based upon the measurement of t_{max} , which is therefore a common point for both the observed and the calculated curves. It is important in this type of study that large saturating doses are not used, so that the responses remain fairly well on the linear part of the dose-response curves.

When the calculated curves are compared with the observed results for glycine and L-glutamate, it is apparent that the diffusion model is

inadequate; the observed decline occurs more rapidly than that predicted. The double-pulse and the steady application experiments described above indicate that 'desensitization' was not occurring. However, the model assumes a first-order receptor activation process in which one molecule of drug reacts with each 'receptor'. If one uses instead the treatment for a *second*-order reaction (e.g. in which two molecules of drug combine with one receptor) then the square of the concentration has to be used in the calculations. The theoretical diffusion curves then come much closer to matching the observed rise and fall of the responses (Fig. 6, filled circles).

DISCUSSION

The results for L-glutamate and glycine suggest that the over-all time course of the response to these iontophoretically applied substances is not greatly influenced by some desensitizing process. Nevertheless, a simple diffusion model based upon a first-order drug-receptor interaction does not satisfactorily describe the response, although it is unlikely that the assumptions concerning the distribution of drug and receptors are entirely adequate (see, for example, the situation discussed by Feltz & Mallart, 1971, in relation to the muscle end-plate). The weakest assumption in the model is that the receptors occupy a single point; in fact the membrane of the Mauthner cell soma and lateral dendrite is fairly uniformly sensitive to GABA and to L-glutamate (and incidentally to glycine also; J. Diamond S. Roper, unpublished observations), and can hardly be envisaged as a 'point receptor' when viewed from distances of less than, say, $100\ \mu\text{m}$ (see Diamond, 1968). However, the results, although more difficult to analyse, were much the same when the pipette tips were far ($\gg 100\ \mu\text{m}$) from the cell. If one assumes, however, that the receptor activation is in fact a *second*-order reaction, the results predicted from the diffusion model more nearly approximate to the observed responses (Fig. 6, filled circles). Higher-order drug-receptor interactions may occur for ACh, GABA, and glycine receptors elsewhere (Takeuchi & Takeuchi, 1969; reviewed by Werman, 1969; see also Katz & Thesleff, 1957*a*); such an assumption is certainly consistent with the results of the present analysis, indicating that the diffusion model may be justified for glycine and for L-glutamate effects at the Mauthner cell. Our results, however, do not exclude the possibility of active uptake or enzyme elimination of these drugs contributing to the rate of decline of their actions, although the relative ineffectiveness of temperature changes on these would not support this possibility.

The frank difference in the time course of GABA responses, along with the apparent 'post-response' depression and 'desensitization' phenomena sets this amino acid apart from the other two studied. It is interesting that

the same slow response to GABA, relative to L-glutamate, has been observed in iontophoretic studies on lamprey spinal neurones (Martin, Wickelgren & Beranek, 1970), at the crayfish muscle (Takeuchi & Takeuchi, 1965), and is an apparent feature of the comparisons of the effects of GABA and glycine on feline neurones (e.g. see Fig. 2 of Curtis, Hösli, Johnston & Johnston, 1968). The difference in the time courses cannot be explained by assuming that at all times the GABA receptor sites were more distant than those of glycine and L-glutamate (cf. Stefani & Gerschenfeld, 1969). It was a consistent finding that despite a very wide range of selected drug-pipette positions, at the soma and out on the lateral dendrite, the responses to GABA were always slower than those to glycine and L-glutamate, particularly when the iontophoretic pipette was near the cell (i.e. closer than 100 μm).

It appears likely that the response to GABA pulses is being limited by some post-synaptic events which might include 'eliminating' systems (e.g. uptake, 'desensitization', etc.). The limitation could also be very dependent on there being a slow dissociation of GABA from the receptors, or on rate-limiting intermediate steps between receptor activation and membrane conductance increases.

It is interesting that the apparent depression of the responsiveness of the Mauthner cell membrane during the declining phase of the response to GABA held not only for a second GABA dose but for glycine as well. This raises the possibility that there may be some interaction between the GABA and glycine receptor-activation. If the initial receptor occupation is the point of interaction, the receptor itself must be complex since its activation by glycine but not that by GABA is prevented by strychnine (see Diamond *et al.* 1973).

However, if both glycine and GABA reacted with identical receptors and the rate of dissociation of GABA was slow compared to that of glycine, it might explain why glycine as well as GABA pulses were less effective during the declining period of a conditioning GABA pulse, and explain too why the converse were not, so i.e. a conditioning glycine pulse would not be expected particularly to influence the accessibility of receptors to the test GABA molecules. If the initial receptors differ, some subsequent step may be common to both amino acids, since the final permeability change are qualitatively indistinguishable (Diamond *et al.* 1973).

The 'depression' which follows a GABA dose may represent some form of inactivating system which accelerates the decline of the response to iontophoretically applied GABA. Speculations have been made that uptake of GABA may terminate its action (Iversen & Neal, 1968). Desensitization of GABA (and glycine) responses has been suggested to occur for mammalian C.N.S. neurones based upon the finding that the response to long iontophoretic applications is not maintained (Dreifuss, Kelly & Krnjević, 1969); however, our experience suggests that one cannot easily rule out changes in drug efflux during maintained constant currents, and it is important in addition to test the effects of consecutive doses of drug.

It is interesting to note that, whatever the nature of this 'depression' process, it is not particularly temperature sensitive (Table 2).

There is nothing in the present results which excludes the possibility of any of the three amino acids being transmitters at the Mauthner cell synapses. The apparent slowness of receptor activation by GABA is a result in part of the relatively sluggish iontophoretic application. We cannot say what the response to GABA might be if the iontophoretic pipette were to be placed right up against the post-synaptic membrane, near to

TABLE 2. The effect of temperature on the shape of the responses to iontophoretically applied drugs. The ratio of the time for the response to decay to $\frac{1}{2}$ its maximum, $T_{\frac{1}{2}}$, to the time to maximum, T_{\max} , is used as an index of the shape of the responses. The temperature of the brain was changed by heating or cooling the perfusion fluid which entered the mouth of the fish

Experiment	Drug	Temperature of the brain (°C)	Ratio, $T_{\frac{1}{2}}/T_{\max}$ (range)
1	Glycine	9	2.5 (2.4-2.6)
	GABA	9	2.2 (2.1-2.4)
	L-glutamate	9	1.9 (1.8-2.0)
	Glycine	18	2.6 (2.3-2.8)
	GABA	18	2.7 (1.8-3.8)
	L-glutamate	18	2.3 (1.8-2.9)
	Glycine	25	2.4 (2.3-2.6)
	GABA	25	2.9 (2.7-3.2)
	L-glutamate	25	2.0 (1.7-2.1)
2	L-glutamate	11	2.1 (1.9-2.5)
3	L-glutamate	11	1.8 (1.7-2.1)
4	L-glutamate	22	2.1 (1.9-3.1)
5	L-glutamate	27	2.7 (2.0-3.7)

Note: the values listed for the ratio are the means of from 3 to 16 measurements with the range given in brackets. The results listed for the first three temperatures (9, 18, 25° C) were made during the same experiment with a triple-barrelled drug pipette, which remained in a relatively stable position during the entire course of the experiment. The remaining L-glutamate experiments (11, 22, 27° C) are different preparations, using a single-barrelled L-glutamate pipette.

a hypothetical 'GABA-nergic' bouton (cf. technique of Harris, Kuffler & Dennis, 1971). However, it is interesting to note that at the Mauthner cell, the i.p.s.p. generated by a single stimulus applied to the inhibitory interneurone is a rather slow and long-lasting event compared to a typical e.p.s.p. (Furukawa & Furshpan, 1963). Furthermore, at known GABA-releasing synapses, for example at the crustacean neuromuscular junction, the i.p.s.p. is also a slower and longer-lasting event than is the excitatory junctional potential (Fatt & Katz, 1953). The slower onset of GABA responses described above for our experiments on the Mauthner cell may

indeed be a characteristic feature of GABA-nergic synapses. At such synapses a tonic inhibitory influence could be achieved by a lower rate of activation than would be needed were the inhibitory endings to cause short-lived post-synaptic responses.

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