PHARMACOLOGICAL REVIEWS
Vol. 17, No. 4

Printed in U.S.A

THE PHARMACOLOGY OF AMINO ACIDS RELATED TO GAMMA-AMINOBUTYRIC ACID

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TABLE OF CONTENTS

I. INTRODUCTION

Although it was generally agreed until comparatively recently that "simple amino acids do not show startling drug actions" (28), there has been a considerable awakening of interest in these substances, particularly because of the neurochemical and neuropharmacological concern with γ-amino-*n*-butyric acid

(GABA) and related compounds (111). Just as it has been rewarding to test the sensitivity of tissues to acetylcholine and catecholamines, and to relate alterations of physiological activity to the function of these substances as synaptic transmitters, many attempts have recently been made to establish a similar physiological role for amino acids.

The first portion of this review outlines reported observations on the effects of simple amino acids on central (Section II) and peripheral (Section III) structures of vertebrates and invertebrates. Section IV deals in more detail with the structure-activity relationships of these compounds as found in those investigations which have been extensive enough to warrant special discussion. The evidence for, and against, a transmitter role for amino acids is presented in Section V, and the possible relevance of these phenomena to various functional states of the mammalian nervous system is discussed in Section VI.

The main emphasis has been placed on GABA, the related dicarboxylic amino acid L-glutamic acid, and amino acids of similar structure which have analogous pharmacological actions. Derivatives of amino acids have been fully discussed only when their actions are probably similar to those of the parent compounds. Substances such as betaines and betaine esters (163, 164, 165) seem to act more on cholinoceptive sites, and have thus been omitted. γ-Aminobutyrylcholine probably also acts on cholinoceptive sites, but as this compound has often been included in studies involving free amino acids, mention is made of its action where appropriate. "Factor I" (107, 116, 117, 118) has not been included as a special topic, as it now seems probable that most of "Factor I" activity is attributable to the amino acid content of the brain extracts in which it is present (107, 223, 231). Nevertheless, due recognition should be made of the historical importance of "Factor I," from the first descriptions of which arose much of the increased interest in the pharmacology of amino acids responsible for the work summarized in this review.

II. CENTRAL EFFECTS

A. Mammalian

Interest in the action of amino acids upon the mammalian central nervous system is closely linked with the possible role of certain of these compounds as central transmitter substances (272). The focal point of attention has been the unique occurrence of γ-amino-*n*-butyric acid (GABA) in this tissue (13, 15, 276, 278, 345; see also 275), together with enzymes associated with its production and destruction (275).

Various methods have been used to administer amino acids to different portions of the central nervous system, and even to single neurones identified by their electrophysiological responses, and it is remarkable that apart from minor differences the findings for any one substance are similar, despite the different techniques which have been used. Although comparatively high doses of amino acids had been shown to induce vomiting in dogs after intravenous administration (166, 282, 346), it was not until the series of experiments by Hayashi and his collaborators (143, 144, 145, 147) that attempts were made to define the central sites of action of amino acids.

All the techniques which have been used to administer amino acids to central structures have disadvantages. For instance, when given intravenously or intra-arterially, these compounds may fail to penetrate to neurones, although the difficulty of the blood-brain barrier may be circumvented either by using immature animals (255) or by making local lesions of the barrier (261). Topical or surface administration suffers mainly from the non-uniformity of action as the applied substance diffuses through various layers of the tissue, and also from the possibility of enzymic alteration of the penetrating amino acid (354). Although localized administration from micropipettes, either by pressure or by electrophoresis (65), is suitable for assessing the amino acid sensitivity of individual neurones, the non-uniform distribution of the substance and the lack of precise knowledge of its concentration in the vicinity of a particular neurone are major disadvantages. Furthermore, the electrophoretic method is limited to substances which are readily ionizable within a limited pH range (77). It is probable that following electrophoretic ejection amino acids rapidly take up an ionic form which depends upon the prevailing tissue pH, irrespective of whether they were ejected as anions or cations (77). Control experiments in which GABA has been ejected either electrophoretically from solutions of different pH (69), or by pressure from neutral solutions (see also 207), do not support the suggestion that the central action of GABA cations or anions differs appreciably from that of the zwitterion (213).

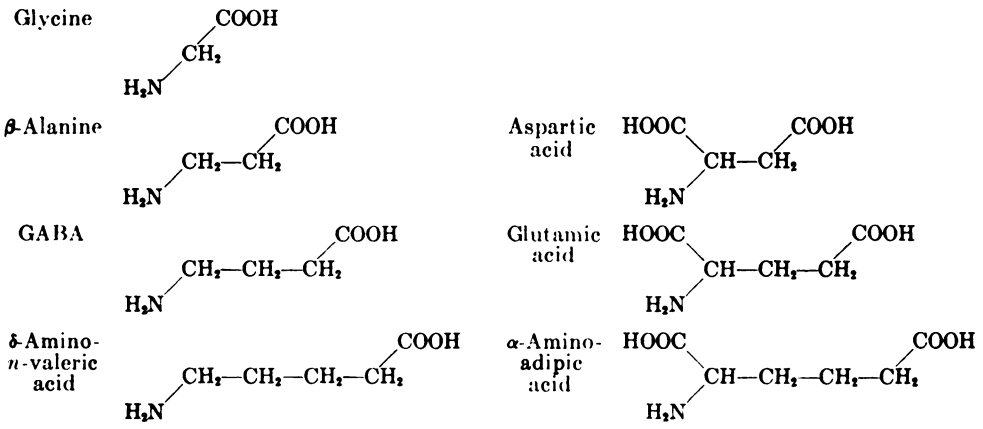

FIG. 1.

1. Cortex. a) Topical and intraventricular administration. There is general agreement that when GABA, or closely related short-chain ω-amino acids such as glycine, β-alanine, δ-amino-*n*-valeric acid (see Fig. 1.) and γ-amino-β-hydroxybutyric acid, in concentrations of 0.0025 M to 1 M, are applied topically to the exposed surface of the cerebral cortex, the negative component of a variety of evoked responses recorded on or just below the surface is reduced or even inverted (39, 47, 78, 131, 139, 161, 172, 175, 192, 230, 232, 242, 251, 257, 260, 265, 309, 314, 316, 382). The effects of these amino acids are readily reversible, and similar observations have been made on the responses of the cerebellar

cortex, although evoked surface potentials are merely decreased and not inverted (97, 259, 260). Short-chain ω-guanidino acids (258, 260, 310), and the sulfur-containing amino acids taurine and 3-amino-1-propane sulfonic acid (78) (see Fig. 2.), also depress cortical responses, although taurine has been reported as inactive (260). γ-Amino-β-hydroxybutyric acid, particularly the L- isomer (148) has been reported to be a more potent depressant than GABA (146, 148, 149, 265). The finding that γ-aminobutyrylcholine is a powerful depressant of cortical function (12, 315, 316) has not gained general acceptance (139, 161), and possibly this substance is pharmacologically active because of its relation to choline, rather than to GABA (160).

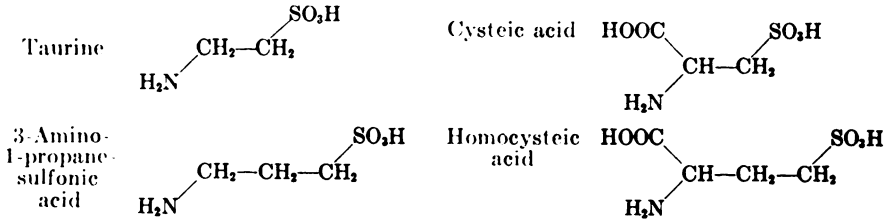

FIG. 2.

When administered into the ventricular system or elsewhere into the cerebrospinal fluid, GABA, taurine and 3-amino-1-propane sulfonic acid depress the activity of cats and mice (59, 135, 139, 178, 260, 352), and raise the threshold to chemically or electrically induced seizures in dogs (146, 148, 149).

Glycine, β-alanine and δ-aminovaleric acid have the same central actions as GABA when administered by intracerebral (presumably intraventricular) injection, but ε-aminocaproic and ω-aminocaprylic acids evoke tonic seizures (135). When applied topically to the exposed cerebral cortex, these longer-chain ω-amino acids and long-chain ω-guanidino monocarboxylic acids augment the negative component of evoked potentials, but have no significant effect on cerebellar cortical potentials (260).

Different interpretations have been placed on the observations that negative cortical potentials are reduced or inverted by GABA, and that cerebellar potentials are merely reduced in amplitude (47, 146, 148, 172, 174, 232, 251, 254, 257, 260, 265), but most of these have not been substantiated by other experimental findings. Amongst other interpretations it has been proposed that GABA is a specific inactivator of excitatory synapses of superficial dendrites (254, 257, 260; but see 172, 174) or a depolarizing agent (47), but it is most probable that this substance, and closely related amino acids, have the same type of action upon cortical neurones (39, 60, 205), as has been proposed for spinal motoneurones (69), namely an increase in membrane conductance without any marked alteration in potential. Furthermore, there has not been any direct experimental verification (207, 209) for the suggestion that long-chain ω-amino acids specifically block inhibitory synapses of superficial dendrites (254, 260).

Many acidic amino acids including glutamic, aspartic, cysteic, α-amino-adipic and related acids, particularly the D- forms of homocysteic and N-methyl-

aspartic acids (see Figs. 1. and 2.), depress cerebral cortical responses and produce spreading depression (78, 260, 354), which is no doubt the consequence of excessive depolarization of nerve cells (see 355). It is of interest that D-glutamic acid is more potent that L-glutamic acid as an inducer of spreading depression in the cerebral cortex of the rabbit, and that L-asparagine (see Fig. 3.) is also very active (354). When introduced into the cerebrospinal fluid, L-glutamic and aspartic acids (146, 330) and related acidic amino acids (59, 193) produce seizures. L-Glutamic acid also depolarizes cells when added to the incubation medium of isolated slices of mammalian cerebral cortex (130, 151), and the threshold concentration has been estimated as 0.2 mM. No such depolarization could be demonstrated for D-glutamic acid (151). DL-Homocysteic, L-cysteic and L-aspartic acids are slightly more potent depolarizing agents than L-glutamic acid, and N-methyl-DL-aspartic acid is much weaker (229).

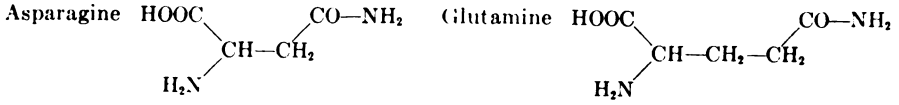

FIG. 3.

b) *Systemic administration.* Although it is generally considered that the blood-brain barrier is relatively impermeable to amino acids such as L-glutamic and GABA (274, 280, 292, 342, 353, 362, 374), and that this explains the relative ineffectiveness of these amino acids upon cortical evoked potentials after intravenous or intraperitoneal injection (135, 260, 330; but see 49), enough of these substances penetrates after intracarotid injection to produce rapid but transient alterations in neurone activity. Thus intracarotid administration of GABA produces a diminution of the surface negative component of cortical potentials (233, 260) and an abolition of cortical seizure activity (146, 148, 316); γ-amino-β-hydroxybutyric acid is more potent than GABA in this latter action (148, 149). On the other hand, L-glutamic acid is a convulsant when injected into the carotid artery (147, 330). Under conditions in which the blood-brain barrier is more permeable, intravenously administered GABA depresses cortical potentials, either in the neonate (255, 256) or after local freezing of the cortex or pretreatment with chloroform-methanol (35, 261). Furthermore, intravenously administered GABA produces weakness and analgesia in cats after prior treatment of the animals with amino-oxyacetic acid ($H_2N \cdot O \cdot CH_2 \cdot COOH$), a finding which together with the histochemical demonstration of GABA transaminase-succinic semialdehyde dehydrogenase activity in the walls of cerebral blood vessels, has led to the proposal that these enzymes could constitute the blood-brain barrier for GABA, and that amino-oxyacetic acid blocks this mechanism (352). The depression of cortical activity by intravenously administered γ-amino-butyrylcholine (12) has not been confirmed by other investigators (139).

In general the influence of orally administered amino acids on the cerebral cortex is minimal (but see 337), although L- and D-glutamic acid, and GABA, increase the motive activity of rats when given by this route (81).

c) *Local administration.* Direct intracortical injections of glutamic acid,

β-hydroxy-glutamic acid, aspartic acid, or asparagine, result in seizures (143, 147, 170, 171, 243), presumably as a consequence of the depolarization of cortical neurones. Injection of these agents into the subcortical white matter is ineffective, although L-glutamic acid induces convulsions when injected into the midbrain (170). Direct injection of GABA solutions into the cortex has been reported not to influence potentials recorded at the site of injection (172).

A more detailed analysis of amino acid action upon single cortical neurones has been carried out by ejection from multibarrel micropipettes, using either current or pressure (60, 205, 206, 207, 208, 262, 302). Structure-activity analyses have demonstrated that the amino acid sensitivity of these neurones is practically identical with that of spinal interneurones (77). Thus amino acids which are structurally similar to GABA, including taurine and 3-amino-1-propane sulfonic acid (60), diminish the sensitivity of cortical neurones to excitant amino acids and block both spontaneous and synaptic excitation, and the more powerful depressants also prevent the invasion of Betz cells by antidromically propagating impulses. Such depression is probably not associated with any change of neurone resting potential (205). It is noteworthy that γ-amino-β-hydroxybutyric acid is somewhat weaker than GABA as a depressant of cortical neurones (60, 207), and that γ-aminobutyrylcholine is a very weak depressant (60).

In contrast, acidic amino acids related to glutamic and cysteic acids depolarize cortical neurones (205), and this excitation is detectable as an increase in firing frequency (60, 207). The threshold concentration for excitation of cortical neurones by L-glutamic acid has been estimated as 0.1 mM (207), and appreciable excitation is obtained with concentrations of the order of 0.5 to 1.2 mM. Although the electrophoretic administration of compounds such as ω-aminocaprylic acid, ε-aminocaproic acid and asparagine does often result in the firing of cortical neurones, the latency of this action is much longer than that obtained with substances more closely related to L-glutamic acid (207), and it is possible that such excitation is not a direct effect of the amino acid molecules (60, 77) (see Sections II A, 2 and IV A, 1).

Cerebellar neurones are also sensitive to amino acids (61, 206, 207, 227); GABA depresses and glutamic acid derivatives excite. Neurones of the pericruciate cortex of newborn kittens have been reported to be depressed by GABA, and excited by L-glutamic acid, although the responses to the latter are said to be relatively poor (208). Neurones of the hippocampal cortex are excited by L-glutamic and DL-homocysteic acid, and depressed by GABA (7, 40, 303).

2. Spinal cord. When applied topically to the feline lumbar spinal cord GABA, γ-amino-β-hydroxybutyric acid (approximately M), γ-guanidinobutyric acid and γ-aminobutyrylcholine (10^{-1} M) do not alter monosynaptic spinal reflexes (161, 230), although higher concentrations of GABA reversibly depress both the patellar and flexor reflexes (38), or flexor reflexes only (18). Both GABA and 3-amino-1-propane sulfonic acid (1 to 2×10^{-2} M) reduce lumbar dorsal root potentials but increase dorsal root reflexes, when applied topically to the appropriate lumbar segments, and occasionally decrease the presynaptic inhibitory action of afferent volleys on monosynaptic reflexes (100). These findings led to the proposal that such neutral amino acids depolarize central terminals of

primary afferent fibres (100). Movements of the hind limbs induced by topical administration of tubocurarine to the upper cervical cord of decerebrate cats are reversibly blocked by GABA (10^{-1} to 1 M) applied in the same fashion (341).

Effects on spinal reflexes have been observed after intravenous administration of GABA. Thus GABA in doses of 0.3 to 10 mg/kg transiently reduces the amplitude of lumbar extensor monosynaptic reflexes, and usually enhances flexor monosynaptic reflexes and polysynaptic reflexes of acute spinal cats (213). Successive doses become less effective, and the action of GABA is not apparent in decerebrate cats (213). The facilitation of flexor and polysynaptic reflexes by GABA is reduced by pentobarbitone, although the facilitation of extensor reflexes is less sensitive to the barbiturate (213). Strychnine reduces the facilitation of the flexor reflexes by GABA, but is without effect upon the action of the amino acid on extensor reflexes. An analysis of the central action of volleys in various afferent pathways, and the effect of GABA on reflexes, suggested that the amino acid blocks excitant interneurones which participate in reflex pathways of high threshold muscle afferents and some cutaneous afferents, and are under control from higher levels (213). Similar effects occur with intravenously administered β-alanine, δ-aminovaleric acid and ϵ-aminocaproic acid, which are however less potent than GABA (239). On the other hand, ω-aminocaprylic and ω-amino-enanthic acids facilitate extensor reflexes and depress the flexor monosynaptic reflexes of spinal cats (214, 239). As with the shorter-chain amino acids these actions are absent in decerebrate cats and the effects on the flexor monosynaptic responses are reduced by strychnine (239); it has been suggested that these longer-chain acids activate certain interneurones. In contrast to the reported failure of GABA to influence spinal reflexes of decerebrate cats (213), comparatively large doses (100 to 300 mg/kg intravenously) initially facilitate spinal reflexes of decerebrate unanaesthetised cats, and then reduce flexor reflexes (18). Intravenously administered GABA does not influence spinal reflexes of acute spinal rabbits, but increases flexor reflexes of rabbits deeply anaesthetised with urethane (322).

An extensive analysis has been made of the action of amino acids ejected electrophoretically upon spinal neurons (69, 70, 77, 78, 79), and all types of cell so investigated, including interneurones, α and γ motoneurones, and Renshaw cells, appear to be equally sensitive to any one amino acid.

GABA and β-alanine reversibly increase the membrane conductance of spinal motoneurones without altering the membrane potential (69). Both inhibitory and excitatory postsynaptic potentials are reduced in amplitude, and antidromic invasion is blocked. The action of GABA is not diminished by strychnine (69), or picrotoxin (80), and it has been proposed that GABA, and closely related substances such as 3-amino-1-propane sulfonic acid, taurine, γ-amino-β-hydroxybutyric acid and δ-amino-*n*-valeric acid, increase the membrane permeability to ions which are normally in electrochemical equilibrium at or near the resting level of transmembrane potential (69). A similar mechanism would account for the observed depression of the spontaneous, synaptic and chemical responses of spinal interneurones, γ motoneurones and Renshaw cells, although the small size of these cells has so far precluded intracellular recording during amino acid

administration. Although GABA and β-alanine fail to modify the amplitude of potentials recorded extracellularly from presynaptic fibres within the spinal cord (69), GABA does depress the electrical excitability of the terminal regions of these fibres (73), where axo-axonic synapses are known to occur (99).

The most active depressants of spinal neurones, in approximate descending order of potency include 3-amino-1-propane sulfonic acid, GABA, β-alanine, taurine, N-methyl-β-alanine, γ-amino-β-hydroxybutyric acid, glycine, α-alanine, δ-aminovaleric acid and β-amino-*iso*-butyric acid. The central actions of longer-chain amino acids such as ω-aminocaprylic acid are somewhat ambiguous, and the failure to reproduce by pressure ejection the delayed excitation obtained after electrophoretic administration indicates that the excitation may not be directly associated with the amino acid molecule (77). γ-Aminobutyrylcholine has little or no depressant effect upon spinal interneurones, but is a strong depressant of the acetylcholine sensitivity and excitant amino acid sensitivity of Renshaw cells and has, in addition, a delayed excitant effect (71, 80). It is probable that these actions stem from its relationship to choline, rather than to GABA (11, 160).

Although the depression of the synaptic responses of spinal interneurones by electrophoretically administered GABA tends to diminish slightly with continued ejection (69), this effect is not always present, is not apparent with β-alanine, and does not necessarily indicate a diminished sensitivity of the cells due to "desensitization" or an increased local inactivation of the amino acid. Despite intensive investigation, no specific GABA antagonist has been found. Substances which have been tested in this respect (80) include N-acetyl-, N-formyl-, and N-lauryl-GABA, imipramine and desmethylimipramine, powerful inhibitors of the binding of GABA by brain particulate fractions (281, 287).

Glutamic, aspartic and cysteic acids reversibly depolarize spinal motoneurones (70, 78, 79), and a similar mechanism explains the firing of smaller cells such as spinal interneurones and Renshaw cells, and the increase in excitability of spinal afferent terminals (73) by these and related substances. Very potent acidic amino acids such as DL-homocysteic acid and N-methyl-D-aspartic acid depolarize motoneurones sufficiently to initiate spike discharges (79). β-N-Oxalyl-L-α,β-diaminopropionic acid, which has been related to neurolathyrism (263), is also a powerful excitant of spinal interneurones and is of similar potency to DL-homocysteic acid (80). Although extensive studies have yet to be reported, the equilibrium potential for the depolarization of spinal motoneurones induced by L-glutamic, DL-homocysteic and N-methyl-D-aspartic acids is at a more polarized level than that of monosynaptic excitation (66). These amino acid excitants do not reduce the effect of subsequently administered L-glutamic or DL-homocysteic acid unless the depolarization approaches a level at which the spike generating mechanism is inactivated (60, 70). Thus an apparent reduction in amino acid sensitivity occurs in association with alterations in the size of extracellularly recorded action potentials (60), but this cannot be ascribed (see 207) to the same type of receptor "desensitization" observed at cholinergic junctions (335).

Despite an extensive examination of a large number of amino acids and other

compounds, many of these bearing acidic and basic groups similar to glutamic acid, as well as other groups of a bulky nature, no specific antagonists have been found for the excitant amino acid action (80). Certain quaternary ammonium compounds sometimes exert blocking actions, but the effect cannot be demonstrated consistently, and its mechanism is in doubt (75). The neutral amino acids related to GABA antagonise the excitant action of acidic amino acids only when an associated membrane conductance change seems probable (see 77).

3. Other central regions. The amino acid sensitivity of other regions of the mammalian central nervous system has not been tested so extensively as have the neurones of the cerebral cortex and spinal cord. Nevertheless, sufficient investigations have been carried out to suggest that all central mammalian neurones have similar sensitivities to these substances. It should be remembered that L-glutamic acid is a comparatively weak depolarizing agent when compared with some of the synthetic amino acids (79), and that when administered electrophoretically it fails to fire large cells such as motoneurones. Hence, reported failures of L-glutamic acid to excite central neurones, when excitation is measured merely by the presence of extracellularly recorded action potentials, do not necessarily indicate that the cells are insensitive to the compound.

Topically administered GABA, β-guanidinopropionic acid and γ-guanidinobutyric acid (approximately 10^{-1} M), but not γ-aminobutyrylcholine, block transmission through the feline nucleus gracilis (161). When administered intracisternally, GABA (5 mg) blocks the flexor movements of the hind limb of the cat induced by electrical stimulation in the contralateral motor cortex, and this depression is prevented by strychnine nitrate (0.15 mg/kg intravenously) (230). An action of GABA upon brain stem neurones, and the possible involvement of the reticular formation, has been proposed to explain the facilitation by intravenously administered GABA of lumbar flexor reflexes in rabbits anaesthetised with urethane, but not in spinal preparations (322), and the desynchronising effect upon the electro-encephalogram of intravenously administered GABA (238, 313). In cats, intraventricular injections of GABA (10 to 20 mg), or topical application to the calamus scriptorius (500 mg/ml), reduce the facilitation of the patellar reflex induced by stimulation of the reticular formation, and the former method of administration also depresses the linguomandibular reflex response to stimulation of the root of the tongue (38). Direct microinjection of GABA solution (10^{-4} M) into the trigeminal and hypoglossal nuclei depresses spontaneous and induced activity of neurones (183). Further evidence for an action of GABA on brain stem neurones is discussed later (Section III A, 4).

When administered electrophoretically, L-glutamic acid excites neurones in the mid-brain and medulla, whereas GABA and β-alanine depress spontaneous and synaptically induced firing (45, 68, 379). L-Glutamic acid and related acidic amino acids also excite neurones of the lateral geniculate nucleus (67, 79), the ventrobasal thalamus (8), and the caudate nucleus (41, 76). It is noteworthy that L-glutamic acid is somewhat more potent than D-glutamic acid as an excitant of thalamic neurones (8), and a similar relationship has been demonstrated for spinal (79) and cortical neurones (60). Only a minority of neurones in the rabbit

olfactory bulb are excited or facilitated by L-glutamic acid (19), but more potent excitant amino acids have not been tested. Ganglion cells of the rabbit retina are excited by L-glutamic acid, and GABA reduces the response of these cells to light (241).

4. Miscellaneous observations. It has been reported that comparatively high doses of GABA, administered subcutaneously (230) or orally (142), and also of L-glutamine and L-asparagine (142) (see Fig. 3.), raise the threshold in mice for seizures induced by strychnine (230), pentamethylenetetrazol and β-methyl-β-ethyl glutarimide (142). However, more recent investigators have been unable to demonstrate antagonism between GABA and these convulsants (48, 110, 135, 225, 260).

γ-Hydroxybutyric acid (215) depresses the activity of cats, dogs, rats and people (90, 216, 217, 378), produces a sleep-patterned electro-encephalogram (90) which differs from that produced by pentobarbitone (378), and reduces spinal polysynaptic reflexes when administered intravenously or topically to the spinal cord (18). These latter effects are antagonised by strychnine (18). The failure of this hydroxy acid to affect the firing of single cortical and spinal neurones when administered electrophoretically (60), together with the latency between systemic administration and the onset of central effects (18, 90), suggests that γ-hydroxybutyric acid has an indirect action upon nerve cell activity. γ-Butyrolactone is also a central depressant (129).

B. Non-mammalian

Potentials recorded in the optic tectum of the seven- to fourteen-day-old chick in response to light are reduced by intravenously (0.8 to 1.3 g/kg) or topically administered GABA (291). Similar observations have been made on the surface negative potentials recorded from the bull frog optic tectum, using GABA or ε-aminocaproic acid, although the latter substance has a preliminary excitant action (296).

The ventral root reflexes of the isolated and perfused frog or toad spinal cord are depressed by GABA (threshold approximately 5×10^{-4} M) and related neutral amino acids (72, 123), and negative potentials recorded from the surface of this tissue in response to afferent impulses are inverted in polarity (296). Atropine, *N-(2-chloroethyl)-dibenzylamine* (Dibenamine), lysergic acid diethylamide, and picrotoxin, but not strychnine, have been reported to reduce the depressant action of GABA on amphibian reflexes (123). A study of a large number of structurally related amino acids has indicated that 3-amino-1-propane sulfonic acid and GABA are the most active depressants (72), whereas longer-chain ω-amino acids (greater than 5 carbon atoms) facilitate spinal reflexes (72, 123). GABA and the sulfonic acid also decrease the amplitude of dorsal root potentials of the isolated toad spinal cord, possibly in association with a slight increase in the excitability of the terminals of dorsal root fibres (290).

L-Glutamic acid (threshold 5×10^{-4} M) and related acidic amino acids depolarize neurones of the toad spinal cord, and under suitable conditions augment reflex transmission (72). Furthermore, L-glutamic acid reduces the

size of dorsal root potentials and depolarizes dorsal root fibres (290). N-Methyl-D-aspartic and DL-homocysteic acids are extremely powerful excitants of this tissue (72), and it is of particular interest that the D- isomers of aspartic, glutamic and N-methyl-aspartic acids are more potent than the L- forms. D- and L-asparagine and L-glutamine are also moderate to weak excitants of the toad spinal cord (72).

It has been reported in a preliminary communication that electrophoretically administered GABA produces a conductance change in the membrane of the goldfish Mauthner cell which is similar to that produced by the natural inhibitory transmitter (87). Although it was proposed that this action of GABA was restricted to areas which are believed to be innervated by inhibitory nerve fibres, namely the axon hillock, cell body and proximal portion of the lateral dendrites, more recent observations (88) indicate that a similar conductance change, which is not readily detectable at the cell body, is produced at more distant regions which are certainly innervated by excitatory nerve fibres. L-Glutamic acid depolarizes the Mauthner cell (87), and sensitivity to this amino acid occurs likewise over the whole membrane.

GABA (3 to 5×10^{-4} M) decreases the membrane resistance of the giant motor fibre of the abdominal nerve cord of the crayfish *Astacus fluviatilis*, and produces a small depolarization of similar order of magnitude to "inhibitory" postsynaptic potentials produced by stimulation of the dorsal surface of the nerve cord (124). The equilibrium potentials for the alteration of potential induced by such stimulation or by GABA are practically identical. On the other hand, similar concentrations of GABA are without action on the lateral giant pre-fibre (124). GABA (10^{-2} M) has no effect upon the fifth abdominal ganglion of the crayfish *Orconectes virilis*, although β-alanine apparently enhances spontaneous activity at low concentrations and blocks at higher concentrations (150). The activity of the cerebral ganglion of the mollusc *Lamellibranchiata* is depressed by GABA (253).

Neurones of the isolated brain of the common garden snail *Helix aspersa* are occasionally sensitive to amino acids (187); the spontaneous firing rate is either increased or decreased by GABA, and the effects of L-glutamic acid are also quite variable (186). Central neurones of *Aplysia depilans* which are hyperpolarized by acetylcholine (H-cells) are usually depolarized by GABA, whereas cells depolarized by acetylcholine (D-cells) are hyperpolarized by GABA (127). Similar differences in amino acid sensitivity have been reported in detail for neurons of *Cryptomphallus aspersa* and *Helix pomatia* (126). Some are depolarized and excited by L-glutamic acid (5×10^{-7} M and above), others are hyperpolarized and inhibited. These changes in potential, produced by either local perfusion or electrophoretic administration, are accompanied by an increased membrane conductance. Although the equilibrium potential for glutamate-induced hyperpolarization coincides with that of the hyperpolarization induced in some cells by acetylcholine, acetylcholine can depolarize other neurones, and there is no relationship between the acetylcholine and L-glutamate sensitivity of different cells (126). Desensitization towards successive doses of glutamic acid

occurs when this amino acid depolarizes the nerve cell membrane, but not when hyperpolarization is produced (126). There is little quantitative difference between D- and L-glutamic acids, and aspartic or cysteic acid produced similar effects. The neutral amino acids GABA, β-alanine and taurine could either depolarize or transiently depress cells excited by dicarboxylic amino acids, temporarily depress cells depressed by glutamic acid, or even have no action on cells sensitive to glutamic acid. Depression induced by GABA is usually transient and not accompanied by a change in membrane potential (126).

The giant nerve cell of the mollusc *Onchidium verruculatum* is unaffected by GABA, β-alanine or γ-amino-β-hydroxybutyric acid (10^{-2} M), whereas γ-aminobutyrylcholine (5×10^{-4} M) mimics synaptic inhibition (136). However, as acetylcholine has a similar action it is considered that the inhibitory effect of γ-aminobutyrylcholine is related more to the choline than to the amino acid moiety (136).

Impulse discharge in the large T fibres of the grasshopper *Gampsocleis buergeri* is depressed by GABA (10^{-1} M) and γ-aminobutyrylcholine, and increased by picrotoxin. These observations, together with certain electrophysiological evidence, have led to the proposal that inhibitory interneurones are present in the prothoracic ganglion of this insect (305). The bioelectric activity of the parasitic infusorium *Opalina ranarum* is depressed by GABA and β-alanine (0.11 to 0.22 M). Depression induced by β-alanine is immediate in onset and recovery is observed despite the continued presence of the amino acid; on the other hand GABA depresses after a latency of 15 to 20 minutes and recovery occurs only after washing out the compound. In contrast, α-alanine increases the activity of this organism (199). The roundworm *Ascarus lumbricoides* is depressed by GABA (4×10^{-5} M) (173), which hyperpolarizes the muscle fibres of the body wall (84).

III. PERIPHERAL EFFECTS

A. Vertebrate

1. Nerve fibre. The literature concerning the action of amino acids on nerve fibre is not very extensive. GABA, γ-amino-β-hydroxybutyric acid and β-alanine (10^{-1} to 1 M) do not depress transmission in feline dorsal or ventral root fibres (69, 230). GABA injected intradermally in people (0.1 ml of M solution), and administered into the conjunctival sac of the rabbit eye (10^{-1} M) has no local anaesthetic effects (156).

L-Glutamic acid in comparatively high concentrations (11 to 55×10^{-3} M) has been reported to depolarize frog motor nerve fibres when applied to single nodes (288), although substitution of almost all of the chloride ions in Ringer's solution by glutamate is apparently without effect on conduction in motor nerves of the frog, *Rana pipiens* (323).

2. Taste receptors. Differences in taste between L- and D-amino acids have been appreciated for some time (see 27, 28), but no systematic study has been carried out relating this to amino acid structure. In general, naturally occurring amino acids are insipid or bitter, whereas D-amino acids are sweet. The "meaty" flavour of L-glutamic acid is of considerable culinary importance.

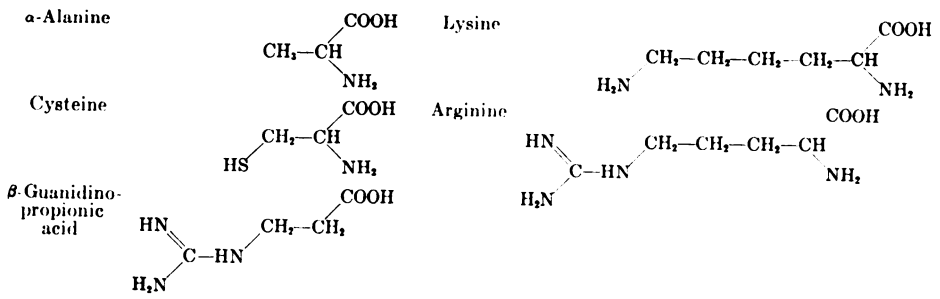

FIG. 4.

3. Autonomic ganglia. Topically administered GABA, γ-aminobutyrylcholine and γ-amino-β-hydroxybutyric acid (10^{-1} M) transiently depress transmission through the feline inferior mesenteric ganglion (161), and a similar result is obtained after intravenous administration of relatively high doses of GABA (100 μg/kg) (234). The superior cervical ganglion of the cat appears to be resistant to GABA given intravenously or intra-arterially (37, 110, 234, 301, 320), although α-alanine (2 to 10 × 10^{-4} M), glycine, lysine, cysteine and L-glutamic acid (3 to 6 × 10^{-4} M) block the excitatory effect of acetylcholine and potassium ions upon this ganglion, and in higher doses block synaptic transmission through it (82) (See Fig. 4.). It has been proposed that the transient hypotension resulting from intravenous administration of GABA or closely related amino acids such as β-alanine, δ-amino-*n*-valeric acid and taurine is associated with a block of peripheral autonomic ganglia in the dog (300, 301).

Methonium compounds do not block the action of injected acetylcholine on the chronically denervated superior cervical ganglion of the cat, and this "effect of denervation" is reversed by D- and L-glutamic acids, D- and L-aspartic acids, D- and L-alanine, β-alanine, L-arginine and L-lysine (248) (see Fig. 4.). Partial explanation of this reversal may be the promotion of the inward transfer of potassium ions into the ganglion cells, and the use of amino acids for oxidative metabolism, rather than a direct action of these amino acids upon external membrane receptors (but see 128).

4. Cardiovascular and respiratory systems. GABA, in comparatively high doses, has no effect upon the isolated perfused rabbit or guinea pig heart, although there is slight enhancement of the force of contraction of the rabbit heart (110). There is, however, no influence on the effects of subsequent doses of acetylcholine or adrenaline. GABA (10^{-6} to 10^{-3} M) has no appreciable action on the isolated hearts of Japanese toads (320); comparatively high concentrations of arginine and lysine depress the isolated frog heart, and histidine stimulates (377). N-Substituted GABA methyl esters have an acetylcholine-like action on the toad atrium (141).

The cardiovascular effects of intravenously administered GABA are exceedingly complex, and there are considerable species differences (37, 110, 300, 301, 318, 319, 320, 322). Cats are less sensitive than dogs and rabbits, and the main effect is hypotension, which is accompanied by bradycardia in dogs. These effects have been attributed to actions of GABA upon peripheral chemoreceptors,

autonomic ganglia (300, 301) or brain stem neurones (37, 319, 320, 322). The latter site of action is suggested by the production of hypotension by GABA administered intracisternally (320), topically to the medulla (37) and by direct micro-injection (0.5 to 1 μg) into medullary areas associated with the central control of blood pressure (320). Amino acids structurally similar to GABA also affect the cardiovascular system, but none is so potent as GABA (301, 318). In contrast, the effects of N-substituted GABA esters resemble those of acetylcholine (312). When administered to anaesthetised vagotomised dogs, taurine depresses the development of adrenaline-induced ventricular premature contractions, and digoxin-induced arrhythmias (264).

Effects of GABA on respiration are also complex (110, 301, 318, 322), possibly because of mixed central effects together with a depression of the sensitivity of pulmonary stretch receptors (89).

5. Neuromuscular junction. GABA (10^{-3} M) has no effect on the contraction of the isolated rat diaphragm (156, 368) and little or no action upon the electrical or acetylcholine sensitivity of this tissue after denervation (368). However, concentrations of 10^{-3} to 10^{-6} M lower both the frequency and amplitude of miniature end-plate potentials recorded from rat intercostal muscle, particularly after pretreatment with veratrine or replacement of the chloride ions in the solution with nitrate (158). The contraction of the frog rectus muscle induced by guanidine is reduced by glutamic acid (0.07 to 0.7 $\times$ 10^{-3} M), glutamine, asparagine (both 7 $\times$ 10^{-3} M), glycine and α-alanine (both 10^{-3} M), although these amino acids fail to influence contractions produced by acetylcholine (85). Similarly, the contraction of this muscle produced by potassium ions is depressed by GABA (10^{-2} M), but there is no clear effect upon the acetylcholine sensitivity (86). Sodium glutamate has been used as an inert substitute for sodium chloride, and is apparently without effect upon the sartorius muscle of the frog *Rana pipiens*, at least in the presence of an elevated calcium ion concentration (323).

6. Gut. The effects of GABA upon the isolated mammalian ileum are complex, vary between species and within any one species, and have been analysed in detail in several publications, which are, however, not in complete agreement (122, 155, 156, 168, 225).

Although GABA (up to 5 $\times$ 10^{-3} M) has little or no effect upon fresh guinea pig ileum (155, 168), both depression and stimulation are observed with "aged" preparations, the stimulant effect being blocked by atropine, N-(2-chloroethyl)N-(2-phenoxyisopropyl) benzylamine (Dibenzyline, phenoxybenzamine) and lysergic acid diethylamide, the depression by atropine and lysergic acid diethylamide (168). The submaximal stimulant effects of acetylcholine, nicotine, histamine (but see 168) and 5-hydroxytryptamine are reduced by GABA (10^{-6} to 10^{-3} M) (122, 155, 156, 168). GABA is more effective as an antagonist of 5-hydroxytryptamine than of the other excitants (156, 168), and resemblances between this antagonism and that exhibited by morphine (156) suggest that the main action of GABA may be upon nervous structures in this tissue, rather than upon smooth muscle fibres. The anti-stimulant effect of GABA is blocked by picrotoxin (168), lysergic acid diethylamide (168), or atropine (155, 168) but not

by strychnine (155, 168), N-benzyl-N′,N′-dimethyl-N-(2-pyridyl)ethylenediamine (pyribenzamine) (168), N,N-dimethyl-N(*p*-methoxybenzyl)-N′-(2-pyridyl)ethylene diamine (mepyramine) or pentamethylene-1:5bis (trimethylammonium) (pentamethonium) (155).

GABA (10^{-6} to 10^{-3} M) also depresses the peristaltic reflex of the guinea pig ileum (155, 168), and this effect is blocked by N-(2-chloroethyl)-dibenzylamine (Dibenamine), lysergic acid diethylamide or picrotoxin (168). An intensive pharmacological study of GABA action upon guinea pig ileum led to the conclusion that this amino acid acted on sites which were closely correlated with tryptamine receptors (168). An analysis of the actions of structurally similar amino acids shows that γ-amino-β-hydroxybutyric acid is less potent than GABA (155, 311), and that β-alanine, glycine, δ-amino valeric acid, ε-aminocaproic acid, α-amino-*n*-butyric acid and β-amino-*iso*-butyric acid are inactive (155, 321). Short-chain guanidino acids have effects like those of GABA (317). L-Glutamic acid (approximately 10^{-3} M) is inactive (155). However, esterification of GABA, with a consequent approach to molecules similar to acetylcholine, enhances stimulant properties (311, 321), and it has been proposed that GABA might compete directly with acetylcholine for receptor sites.

Although the effect of GABA and related amino acids upon cat ileum is similar to that upon guinea pig ileum (321), rabbit ileum (122, 155, 156, 321), rat ileum (155) and rat duodenum (156) are less sensitive to these agents. Furthermore, GABA shows little or no antagonism towards the stimulant effect of 5-hydroxytryptamine upon rat or guinea pig uterus (156).

B. Invertebrate

1. Nerve fibre. Although it has been reported that GABA (10^{-5} M) irreversibly increases the threshold of crab nerve (185), GABA, in concentrations 1000 times that required for block of the crustacean stretch receptor (*i.e.*, 1000×10^{-5} M), has little or no effect upon the sensory axons of the lobster (212). GABA (10^{-4} to 10^{-2} M) (95, 269) and β-guanidinopropionic acid (10^{-2} M) (95) have no action upon crayfish nerve fibres, although lower concentrations (10^{-5} M) readily block neuromuscular excitatory transmission (93, 95, 269). Peripheral nerve fibres of the crayfish (*Cambarus clarkii*) are also insensitive to L-glutamic acid (up to 25×10^{-3} M) (269, 354, 357).

2. Chemoreceptors. An analysis has been made of the amino acid sensitivity of dactyl chemoreceptors of *Carcinides maenas* by recording from the appropriate sensory nerve fibres (52). These chemoreceptors are particularly sensitive to L-glutamic acid (1 to 5×10^{-5} M) and less sensitive to the D- isomer; in some cases the two enantiomers may activate different sensory receptors. Differences in activity can also be detected between D- and L-aspartic acid, and between D- and L-leucine; the activities of these and other amino acids including glutamine and GABA are much less than that of L-glutamic acid. The dactyl chemoreceptors of other brachyurans are also very sensitive to L-glutamic acid (53), although receptors of the walking legs of *Carcinus maenas*, *Portunas puber* and *Homarus vulgaris* are insensitive to the amino acid, and also to aspartic acid, glycine,

glutamine and GABA (221). Chemoreceptors of the antenule of *Cambarus bartonii* are sensitive to L-glutamic acid (157), whilst those on the walking legs of the arthropod *Limulus polyphemus* are excited by glycine (10^{-2} to 10^{-3} M) (16).

3. Stretch receptors. GABA (10^{-4} to 10^{-5} M) reduces the stretch-induced discharge of the abdominal and thoracic stretch receptors of a variety of crustacea (25, 101, 102, 106, 112, 115, 120, 123, 137, 211, 212, 228, 230, 375). A particularly close analysis has been made of the effect of this amino acid upon the slowly adapting stretch receptors of crayfish and lobsters (137, 212). GABA induces an increase in membrane conductance which resembles closely that produced by stimulation of inhibitory nerve fibres terminating upon the dendrites of these stretch receptors. The possibility that GABA is the actual inhibitory transmitter is also suggested by the finding that picrotoxin blocks both neural inhibition and the effect of GABA (123, 211). However, stretch receptor cells devoid of inhibitory synapses are also depressed by GABA (212) and hence the specificity of this amino acid for inhibitory synapses is questionable, although radioautographic studies indicate that the site of action is mainly at the axodendritic nerve endings between branches of the inhibitory axon and stretch receptor dendrites (298).

Detailed analyses have been made of the relationship between amino acid structure and effectiveness as depressants of crustacean stretch receptors (101, 103, 115, 123, 137, 212, 228; see Section IV B, 1). Evidence was obtained that some amino acids were inactivated in the vicinity of the crayfish stretch receptor, transient depressions being restored by stirring, whereas the transient affects of other amino acids resulted from desensitization (103). Neither the inactivation of GABA nor the inhibitory synaptic potential is altered by amino oxidase inhibitors (103). γ-Aminobutyrylcholine excites crayfish stretch receptors (137) in the same way as acetylcholine does. The stretch receptors of the silkworm *Bombyx mori* are insensitive to GABA (200).

L-Glutamic acid, L-aspartic acid, L-asparagine and L-glutamine (approximately 10^{-2} M) do not influence the activity of stretch receptors of the crayfish *Cambarus virilis* (109), although a later report indicates that stretch receptor activity may be enhanced by L-glutamic acid (112). This excitation is followed by a depression of firing similar to that obtained with GABA; desensitization is observed with repeated applications of L-glutamic acid (112). L-Aspartic acid has an action similar to that of GABA on the abdominal stretch receptors of *Pacifastacus leniusculus* (120). γ-Amino-β-hydroxybutyric acid trimethyl betaine (carnitine) (103) and γ-butyrobetaine (115) are also excitants of stretch receptors, presumably because of the resemblance to acetylcholine (109).

4. Crustacean heart. GABA (10^{-5} M) inhibits the heart of the crayfish *Astacus trowbridgii*, and this effect is blocked by picrotoxin, as is cardiac inhibition produced by nerve stimulation (117). The action of GABA on the lobster (*Homarus*) cardiac ganglion is also similar to stimulation of cardio-inhibitory nerves (235). A range of amino acids has been tested on the perfused lobster heart (*Homarus americanus*), of which GABA is the most potent depressant (113). Aspartic and glutamic acids stimulate this preparation; D- and L-glutamic

acid are equally potent, but less active than aspartic acid. Asparagine is inactive (113). However the perfused crayfish heart (*Cambarus virilis*) is depressed by L-glutamic acid (12 to 25 $\times$ 10^{-5} M) and excited by L-aspartic acid (10^{-3} M) (48).

5. Muscle. The contraction of many types of crustacean muscle in response to stimulation of the excitatory nerve is depressed by GABA in comparatively low concentrations (10^{-6} to 10^{-3} M) (5, 42, 48, 96, 105, 112, 121, 134, 230, 246, 269, 325, 350), although GABA has been reported to have no effect upon muscle fibres of some crabs and crayfish (167). GABA increases the membrane conductance of fibres of the opener muscle of the claw of the crayfish *Astacus fluviatilis* (42), of the lobster *Homarus americanus* (134), of the crayfishes *Cambarus clarkii* (246, 325, 350), *Orconectes immunis* (350) and *O. virilis* (93, 96, 246, 350), and of the crab *Cancer borealis* (105); but the conductance change induced in the fibres of the crabs *Cancer magister* (121) and *C. borealis* (5) is minimal. However, there is evidence for two types of muscle fibre in muscles of *Cancer magister*, only one of which is very sensitive to GABA (14).

The increase in membrane conductance of crustacean muscle fibres is associated with a diminution in the magnitude of postsynaptic junction potentials, and an alteration in membrane potential which is practically identical to the inhibitory junction potential (42, 93, 134, 211, 325, 350). It is thus probable that both GABA and the inhibitory transmitter induce the same conductance increase in junctional membrane, namely an increase in chloride permeability (42, 134). Approximately 4 $\times$ 10^{-15} mole of GABA is required by local microelectrophoretic ejection to produce a potential of the same size as the inhibitory junction potentials recorded intracellularly from fibres of the crayfish *Cambarus clarkii*, and the sites of maximum sensitivity to GABA correspond to inhibitory neuromuscular junctions (325). GABA is inactive when ejected electrophoretically into the interior of these muscle fibres, and although the sites of maximum sensitivity on the external surface correspond to those sensitive to L-glutamic acid, it proved possible to desensitize the muscles to L-glutamic acid, by prolonged administration, without affecting the sensitivity to GABA (325). Thus different membrane receptors are presumably involved for these two amino acids which have opposite effects (see below). For most crustacea, picrotoxin is an antagonist of both the action of GABA and synaptic inhibition (93, 134, 270, 349, 350), although picrotoxin is not a GABA antagonist in the crab *Cancer magister* (121).

Evidence has also been obtained that GABA increases the membrane conductance of the terminals of excitatory nerve fibres of the crayfish (*Orconectes virilis*, *Astacus fluviatilis*), with a consequent reduction in the amount of transmitter released (91, 93, 95, 96), and the mechanism is indistinguishable from that of neural inhibition (92, 94). β-Guanidinopropionic acid has a similar presynaptic effect which, like that of GABA, is blocked by picrotoxin (93). In contrast to GABA, however, this guanidino acid does not increase the conductance of the postsynaptic muscle membrane (91, 93, 95); yet it reduces the postsynaptic effectiveness of GABA and the inhibitory transmitter (93). A presynaptic action

of GABA has also been proposed for the lobster (133) and the crab *Cancer borealis* (5).

A considerable number of neutral amino acids has been tested for depressant actions upon crustacean muscle fibres (93, 95, 134, 230, 269, 350); GABA is the most potent and activity falls with lengthening and shortening of the carbon chain (see Section IV B, 2). Some guanidino acids also depress excitatory synaptic transmission in the crayfish (93, 269), but only guanidino-acetic acid has a postsynaptic effect similar to that of GABA; other guanidino acids increase the membrane conductance of excitatory presynaptic terminals only (93).

GABA mimics the action of the peripheral inhibitory transmitter upon muscles of the grasshopper *Romalea microptera* and the locust *Schistocerca gregaria*; the GABA effect is blocked by picrotoxin (347).

In contrast to the depression of crustacean muscle by GABA, comparatively low concentrations of L-glutamic acid reversibly depolarize and give rise to contractions (42, 112, 267, 269, 324, 354, 355, 357). Concentrations of the order of 2 to 4 $\times$ 10^{-5} M contract the muscle of the crayfish *Cambarus clarkii* (269, 354, 357), and when administered electrophoretically approximately 4.5 $\times$ 10^{-16} mole (producing a local concentration of 4 $\times$ 10^{-5} M) produces an appreciable depolarization (324). L-Glutamic acid is inactive when ejected into the interior of crayfish muscle fibres, and probably has no action upon presynaptic structures (324). Maximal sensitivity of the external surface of the muscle membrane corresponds with the sites of excitatory synapses, but it has not been possible to compare equilibrium potentials for synaptic excitation and glutamic depolarization (324). In the continued presence of L-glutamic acid the membrane becomes less sensitive to both L-glutamic acid (112, 269, 324, 357) and the excitatory synaptic transmitter (269, 324; see also 48, 357); prior to this desensitization the membrane is apparently more sensitive to both the amino acid and the transmitter (324). In high concentrations (5 $\times$ 10^{-4} M) L-glutamic acid reversibly blocks excitatory neuromuscular transmission (48, 269).

It has been proposed that L-glutamic acid interacts with the receptors for the excitatory synaptic transmitter and mimics its action (269, 324, 355, 357). GABA blocks the excitation of crustacean muscle by L-glutamic acid (48, 269, 357), presumably because of the conductance change induced at inhibitory synapses since there is evidence that the two amino acids interact with different receptor sites (325). Picrotoxin is not an antagonist of L-glutamic acid (269).

Crustacean muscle is very insensitive to D-glutamic acid (269, 324, 354) and the ratio of the potencies of the two isomers has been given as approximately 250 to 500 (354). The local electrophoretic administration of the D- isomer does not influence the muscle sensitivity to simultaneously administered L-glutamic acid (324), although high concentrations (above 10^{-3} M) block the contraction of crayfish muscle induced by stimulation of the excitatory nerve without any prior facilitation or contracture (269). L-Glutamine is approximately one-seventieth as active as the parent amino acid and is more potent than L-aspartic acid (354). The latter amino acid has been reported to block transmission at the excitatory neuromuscular junction of the crayfish *Orconectes virilis* (203). Other

dicarboxylic α-amino acids are without effect upon crustacean muscle fibres (269, 354).

L-Glutamic acid is an excitant of the pharyngeal retractor muscle of the snail *Helix aspersa* and limb muscles of the cockroach *Periplaneta americana* (threshold concentrations approximately 10^{-6} M); the snail muscle is 40 to 100 times more sensitive to L-glutamate than to the D- isomer (184).

6. Gut. GABA (5×10^{-4} M) contracts the isolated squid rectum, and γ-amino-β-hydroxybutyric acid is less active (230). The sea urchin oesophagus is insensitive to GABA (10^{-3} M) (122). In contrast, the spontaneous and acetylcholine-induced contractions of the isolated hind gut of the crayfish (*Cambarus clarkii*, *Orconectes virilis* and *Pacifastacus leniusculus*) are depressed by GABA (2×10^{-5} M), γ-amino-β-hydroxybutyric acid, β-alanine, taurine, L-glutamic acid (3×10^{-4} M) and L-aspartic acid (119, 120). The action of these depressants is blocked by picrotoxin (119). When the extracellular sodium concentration is lowered, GABA, L-glutamic acid and a series of related amino acids contract the crayfish hind gut (194). The isolated hind gut of the European crayfish *Astacus fluviatilis* contracts in the presence of L-glutamic acid (3×10^{-5} M), D-glutamic acid being ten times less active (180). Other closely related amino acids also cause contractions.

IV. STRUCTURE-ACTIVITY RELATIONSHIPS

A. Vertebrate

1. Single neurones. The structural determinants of amino acid excitant and depressant action on mammalian central neurones have been extensively investigated by microelectrophoretic application of the substances into the environment of single cells within the spinal cord and brain of the cat (see Sections II A, 2, and II A, 1 c) (60, 77, 78, 79, 207). There seems to be little variation in the structure-activity relationships with the different types of central neurone upon which they have been determined, although the conclusions reached in two separate studies of cerebral cortical neurones differed in certain minor respects (60, 207). The main findings can be summarized by reference to the general structure below.

$$Y \cdot (CH_2)_n \cdot \overset{*}{\underset{}{C}}H(X) \cdot NH_2$$

For an amino acid to be a strong depressant, X = H, Y = CO_2H, SO_2H or SO_3H; n = 1 to 3. Substituents on carbon atoms within the chain, or on the nitrogen atom, reduce or abolish depressant activity, according to their position, size and number. The acidic and basic groups must be free, that is, not involved in ester or amide linkages. Phosphonic and boronic analogues of depressant amino acids [Y = $PO(OH)_2$ or B $(OH)_2$] are weak or inactive (80), as are also certain phosphate esters of ethanolamine (54). The guanidino group, .NH.CH(:NH).NH_2 may replace the amino group at the end of the carbon chain provided the latter is somewhat shortened (60, 207). Stereospecificity has not been sys-

tematically investigated amongst compounds existing in different optical forms. The most potent amino acid depressants in the case of the feline central nervous system are GABA and its sulfonic analogue, 3-amino-1-propane sulfonic acid, $HO_3S.(CH_2)_3.NH_2$. The latter is several times more active than the former (60, 78).

For an amino acid to be a strong excitant, X = CO_2H, Y is again CO_2H, SO_2H or SO_3H, and n = 1 or 2. ω-Phosphonic and ω-boronic analogues of the excitant amino acids [Y = $PO(OH)_2$ and $B(OH)_2$, respectively] are weak or inactive (80). Whether X can be an acidic group other than carboxyl has not been investigated. The starred carbon atom being asymmetric, there exist D- and L- series of excitants. The structure-activity relationships found for the L- series are practically identical with those found for the depressants. Thus, alkyl substitution within the carbon chain, or on the nitrogen atom, reduces activity to an extent dependent upon the size, position and number of substituents, and both acidic and basic functions must generally be free (60, 77, 79). Although electrophoretically administered L-asparagine has an excitatory effect (60, 207), the failure to reproduce this delayed excitation by pressure ejection raises doubt as to a direct action of the molecule (60, 77). With certain important exceptions, D- and L-forms of the one excitant do not differ markedly in potency from one another, and although differences have been reported, particularly with D- and L-glutamate (207), these can be ascribed to other factors (60). The important exceptions are the enantiomers of homocysteic acid, $HO_3S.(CH_2)_2.CH(NH_2).CO_2H$, and those of certain N-alkyl aspartic acids, $HO_2C.CH_2.CH(NHR).CO_2H$ [R = CH_3, C_2H_5, CH:NH or n-C_3H_7], in all of which pairs the D- form is very much stronger than the L- form (77, 79). The D-aspartic acid derivatives are exceptional in another respect. This is the only set of compounds amongst various carboxylic, sulfonic and sulfinic acids, of both D- and L- series, where N-alkylation has resulted in a marked increase, rather than the usual marked decrease in potency. The strongest excitants yet found are N-methyl-D-aspartic acid and D-homocysteic acid (79). The acidic amino acids occurring in nervous tissue, L-glutamic, L-aspartic, L-cysteic (328) and L-cysteine sulfinic acids (29) do not differ markedly in potency, and all are of moderate strength (79). The syntheses of a large number of excitatory amino acids have been described (54, 366).

A recent finding which is extremely interesting, both because of its implications with respect to structure-activity relationships, and because of the possible relationship to neurolathyrism (see 294), is that β-N-oxalyl-L-α,β-diaminopropionic acid is a potent excitant of spinal neurones (80). This compound, isolated from the seeds of *Lathyrus sativus* (263) has been claimed to be responsible for the neurological manifestations of lathyrism. It has the formula $HO_2C.CO.NH.CH_2\cdot CH(NH_2).CO_2H$, and in chain length resembles α-amino-adipic acid (which is only a very weak excitant) more than glutamic acid. Yet the lathyrus compound is several times more potent than L-glutamic acid, and is the strongest L-amino acid yet tested. Its potency is somewhat lower than D-homocysteic acid, and about equal to that of N-ethyl- and N-iminomethyl-D-aspartic acids (79). The only similar compounds which have so far been tested are γ-N-oxalyl-α,

γ-diaminobutyric acid, N-oxalylglycine and N-oxalyl-β-alanine, all of which are inactive (80).

2. Whole tissue. To a large extent, the structure-activity relationships observed in the above investigations on single cells apply also in the case of amino acid excitants and depressants applied topically to the cerebral (78, 254, 260) and cerebellar cortices (254, 260) (see Section II A, 1 a). Because of the difficulties in interpreting results obtained with preparations involving a multiplicity of interconnected cells (72), only the major differences under the two sets of conditions will be described.

Although D-glutamic acid initiates spreading depression at a lower concentration than the L- isomer when administered topically to the rabbit cortex (354), the latter amino acid is a more potent excitant when administered electrophoretically to single neurones in the cat cerebral cortex (60, 207). The comparatively high potency of the D- isomer in initiating spreading depression has been ascribed to the metabolic inactivation of L-glutamic acid by cortical tissue (354), and corroborative evidence has been provided (252).

Topically administered L-asparagine is a notable exception to the general rule found in electrophoretic studies that the acidic and basic functions of both depressants and excitants must be free. L-Asparagine has a quite marked effect in producing spreading depression when applied to the cerebral cortex of the cat (254, 260), and rabbit (354). L-Glutamine has a much weaker action in both cases. The potency of L-asparagine is very similar to that of L-aspartic and L-glutamic acids, which undoubtedly initiate spreading depression by a direct depolarizing effect on the cortical neurones. It is possible that the action of the amide is due to its extraneuronal enzymic conversion into the free acid, but the rapidity of the action, and the fact that the amide is, in fact, slightly more potent than the free acid on the rabbit cortex (354) do not support this hypothesis. Although cat cortical and spinal cells can be made to fire paroxysmally by prolonged electrophoretic ejection of asparagine into their environment (60, 207), this effect has been ascribed to changes in the extracellular hydrogen ion concentration inherent in the method of administration, and not to asparagine itself (60). There is some doubt, therefore, that cortical cells are depolarized by the direct action of asparagine, and in the absence of such a direct effect, the cause of the spreading depression which follows the topical application of this substance to the cortex may lie in redistribution of amino acids or ions resulting from its uptake into the tissue.

The other main difference between the results obtained with the two methods of administration is the apparent excitatory action of long-chain ω-amino and ω-guanidino acids on the cerebral cortex, attributed to a block of superficial dendritic inhibitory synapses (132, 254, 260), as contrasted with the lack of activity (except such that could be ascribed to other causes) which these substances seem to have on single cells (77). The situation is similar to that of asparagine in that, although cells in the cortex (60, 207) and spinal cord (77, 80) can be excited by the electrophoretic administration of substances such as ω-aminocaprylic and ω-aminononanoic acids, this excitation cannot be repro-

duced by pressure ejection of these amino acids from micropipettes (60, 80). The excitation demonstrated electrophoretically can be explained by a lowering of the pH of the extracellular fluid due to release of protons from the amino acid cations (77), but other factors may be involved. The effect which follows topical administration of the long-chain ω-amino acids to the cerebral cortex is quite different from that of acidic amino acids such as aspartic and glutamic acids, and also from that of asparagine, all of which produce spreading depression as a result of a more drastic permeability change of the neuronal membranes. The action of the long-chain compounds may be a result of inhibition of enzyme systems controlling the ionic permeability of membranes, the extracellular levels of endogenous active substances, or both. Such actions are not likely to be detected by the electrophoretic method, which in general involves administration for brief periods only and would rarely result in concentrations high enough to imfluence all but the most susceptible enzyme systems (70). An alternative explanation of the effect is that these substances, having both hydrophilic and hydrophobic components, are likely to be surface-active, and, at the concentrations used for topical administration, are possibly capable of nonspecific penetration of critical regions of the neuronal membrane, with resultant changes of membrane permeability. However, the absence of any demonstrable effect of ε-aminocaproic, ω-aminocaprylic and ω-aminononanoic acids upon the evoked potentials of the cerebellar cortex (254, 260) renders difficult any interpretation of the action of such substances which does not involve differential effects on different types of neurone or synaptic process. Whatever the mechanisms of the excitatory effects of these long-chain compounds, they clearly differ from those of the short-chain amino acids.

Two brief surveys of structure-activity relationships with respect to amino acids injected intraventricularly into mouse brain have been described (59, 193) (see Section II A, 1 a). Those substances found to be the strongest excitants and depressants when administered electrophoretically around single cells are also the most potent convulsants and depressants, respectively, following intraventricular injection. N-Methyl-D-aspartic acid (59, 193) and D-homocysteic acid (59) are the strongest convulsants, and 3-amino-1-propane sulfonic acid and GABA (59) are the strongest depressants. The other substances tested followed the same general pattern as described for single cells.

A detailed study of the structure-activity relationships of amino acid action on the isolated hemisected toad spinal cord has been made (72) (see Section II B). The results are very similar to those obtained in mammalian cortex experiments which involved topical application of many of the same substances (132, 254, 260). Again there are certain exceptions to the general structure-activity "rules" for amino acid action as applicable to single neurones of the mammalian central nervous system (77). Thus D-asparagine (ω-carboxyl replaced by amido group) D-malic acid (α-amino replaced by hydroxyl group), D-serine (ω-carboxyl replaced by hydroxyl group), and long-chain ω-amino and ω-guanidino acids are all excitatory, these actions contrasting with the apparent inactivity of the substances on cat spinal neurones (77). The relative potencies of other excitants and

depressants fairly closely parallel those found for the same substances on individual neurones of the cat central nervous system. It could be argued that all of the exceptional effects have different mechanisms from that responsible for, say, aspartic acid excitation, particularly in the case of the long-chain compounds (see above). The short-chain compounds, however, are all closely related structurally to aspartic acid, and the time courses of their actions are similar to that of aspartic acid. Moreover, it is noteworthy that the D- forms of the three exceptional short-chain compounds are markedly more potent that the L- forms, as is the case also with the enantiomers of both aspartic and glutamic acid (72). This fact could well be explained by a more rapid inactivation of the L- form by extracellular enzyme systems, as proposed for glutamate-induced spreading depression of the cerebral cortex (252, 354), but it should be pointed out that the L- forms of certain synthetic amino acids, which are unlikely to be rapidly inactivated by enzymes, are also markedly less potent than the corresponding D- forms on single neurones of the cat nervous system (see section IV A, 1). Hence the observed enantiomeric effects on whole tissue may reflect valid differences in excitatory potency. If this is so, the fact that the D- forms of aspartic acid, asparagine, serine and malic acid are the strongest enantiomers in each case suggests a similarity in mechanism between the actions of the exceptional and the normal excitants, and it remains possible that the failure to demonstrate similar actions on single neurones of the mammalian nervous system merely reflects a species difference in potency. Two other, less detailed, investigations of the effects of amino acids on the responses of amphibian spinal cord (123, 296) have also been carried out, the results in general being in line with those already mentioned.

B. Invertebrate

1. Crustacean stretch receptor. The blocking actions of short chain ω-amino acids on the crayfish stretch receptor are not greatly different from those found on mammalian and amphibian neurones, to the extent that they have been studied (103) (see Section III B, 3). There is a sharper peak at $n = 3$ (GABA) in the series of varying chain length, and also ω-guanidino compounds are markedly more potent, than in the case of the various vertebrate test systems. Sulfonic and sulfinic acids have not been studied in detail, but taurine is very weak. Long-chain ω-amino acids appear to be inactive (103).

2. Crustacean neuromuscular junction. Short-chain ω-amino monocarboxylic acids reversibly depress, and short-chain ω-amino dicarboxylic acids reversibly excite, crustacean muscle (see Section III B, 5). The structure-activity relationships determined for depressant action (see particularly 93) are similar to those determined on the crayfish stretch receptor except that taurine (269) appears to be more active than in the latter case, having about the same potency as β-alanine (one-fiftieth of that of GABA—269). Long-chain ω-amino acids are inactive (269). The situation regarding guanidino acids is rather complex (93) and suggests that in the crayfish, the pre- and postsynaptic amino acid receptors are not identical, although picrotoxin appears to be an antagonist at both sites.

Guanidino-acetic acid is almost as potent as GABA (93, 269) and has an identical postsynaptic effect (as in the lobster, 134). β-Guanidinopropionic, β-guanidinobutyric, α-guanidinobutyric and γ-guanidinobutyric acids do not increase the postsynaptic membrane conductance (93), but do have presynaptic effects identical with that of GABA. However β-guanidinopropionic acid must interact with postsynaptic receptors since it reduces the effectiveness of both GABA and the inhibitory transmitter upon the postsynaptic membrane (93).

Amongst the stimulant series (269, 354, 357), L-glutamic acid is the most potent, activity falling off markedly as the chain length is varied. D-Glutamic acid is much weaker than the L- form. L-Glutamine and L-aspartic acid (although 70 and 100 times weaker, respectively) are amongst the next most potent excitants after L-glutamic acid, for which the preparation shows a remarkable specificity. However, the series of compounds tested is not extensive and does not include any of the substances found to be very powerful excitants of neurones in the mammalian central nervous system.

3. Crustacean gut. A series of amino acids has also been tested on the responses of the hind gut of the crayfish (see Section III B, 6). GABA and guanidino-acetic acid are the most powerful depressants of acetylcholine-induced contractions of the hind gut of some species (119), while substances such as β-alanine and taurine are 50 to 100 times weaker. L-Glutamic acid, L-aspartic acid, and various hydroxylated derivatives of these two substances cause contractions of the hind gut of *Astacus fluviatilis* in low concentrations (180). D-Glutamic acid is about one-tenth as active as the L- form, and L-glutamine and L-asparagine are inactive at 40 times the threshold concentration for L-glutamic acid (180).

V. PHYSIOLOGICAL ROLE OF EXCITANT AND DEPRESSANT AMINO ACIDS

Where a particular tissue has special mechanisms for elaborating considerable quantities of substances which, when released into the extracellular environment, exert a significant influence on individual cells, it is reasonable to enquire whether the substances may be involved physiologically in the control of cellular activity. This situation exists in the case of the neuropharmacologically active amino acids occurring chemically free in large abundance in nervous tissue (327). It is thus important to discuss the sites upon which the amino acid actions are exerted, together with the mechanisms of these actions, and to draw parallels, where they exist, with known physiological events.

Several possibilities exist as regard the sites of action. These include: 1) junctional "receptors" specialized for combination with synaptic transmitter substances; 2) membrane components concerned with the general maintenance of intra- and extracellular ionic concentrations; 3) membrane components involved in active and passive mechanisms for the accumulation and control of amino acids for metabolic processes, and 4) structural components not subserving any special function, other than that of conferring mechanical strength on the membrane.

There exists no experimental evidence as to the molecular nature of the amino acid receptor sites, but it can be inferred that these are situated on the outside

surfaces of the cells which are sensitive to the compounds (70, 324, 325). In view of the different sites of action which can be demonstrated in some cases on crustacean muscle (325), the antagonism of GABA but not of L-glutamate by picrotoxin (269), and the fact that the excitant amino acids have apparently little effect on the crayfish stretch receptor (109, 112), it would seem likely that the excitants and depressants act on different membrane components. However, the possibility must not be excluded that, at least at some sites, membrane steric features allow the attachment of both types of amino acid to the one molecular component, such interaction resulting in the different permeability changes characteristic of the two types of action (77, 367). General features of amino acid receptor sites on neurones have been discussed (72, 77; see 169) and a molecular theory of amino acid action involving membrane phospholipids has been proposed (367).

In certain cases distinctions can be drawn between the various possible sites of action, and this evidence can be coupled with that relating to the ionic mechanisms underlying the observed pharmacological effects, and also with metabolic findings, to present a strong case for amino acid involvement in physiological processes concerned with the control of nervous activity. In the following sections, the problem will be considered in some detail, firstly as it relates to crustacea, on which much definitive work has been carried out, and secondly, with regard to mammals, for which the evidence is more circumstantial, but of prime importance from the point of view of normal and abnormal human nervous function.

A. Invertebrate

The action of GABA seems to be restricted to junctional regions of membrane. Thus, GABA-sensitive regions of crustacean muscle are coincident with end plates (325), and GABA is bound more in regions of high density of inhibitory synapses than elsewhere on crayfish stretch receptors (298). Sensory and motor axons are not blocked by high concentrations of GABA (212, 269). Neural inhibition and GABA-induced depression are caused by membrane conductance changes which seem to involve very similar ionic mechanisms for inhibitory synapses on crustacean stretch receptors (101, 103, 137, 211, 212), crayfish giant motor axons (124) and crustacean muscle (14, 42, 134, 325); and both effects are blocked by picrotoxin in each case (134, 270, 349, 350). The ionic conductance changes induced in the postjunctional membrane in these tissues seem to involve mainly an increase in chloride ion permeability of the affected regions, although potassium ion may also be involved to some extent (42, 212). GABA exerts differential effects on tonic and phasic fibres of crab muscle in a manner dependent upon the chloride permeability and chloride equilibrium potential of the membrane, in much the same way as the neural inhibitory transmitter liberated at inhibitory terminals (14). Presynaptic inhibitory effects in crustacean muscle are also simulated closely by GABA (95, 96).

GABA occurs in inhibitory fibres and cell bodies of the lobster in much higher concentration (approximately 0.1 M) than in excitatory motor fibres (approx-

imately 0.001 M), excitatory cell bodies and sensory fibres, and although several other blocking substances also occur, these are weaker depressants than GABA and are not likewise asymmetrically distributed between inhibitory and other fibres (202, 203, 204, 247). Although glutamic acid and glutamic acid decarboxylase occur in both excitatory and inhibitory nerve fibres (201, 204), decarboxylase activity is much higher in the latter than in the former (138). Thus the evidence is very strong that GABA is an inhibitory transmitter in these crustacea, particularly at the neuromuscular junction (but see 120). However, before a final conclusion is reached to this effect, it will be necessary to demonstrate the release of GABA from presynaptic inhibitory terminals in the quantities necessary for inhibitory action. Until then some reservations about its role should be held, particularly in view of the fact that GABA causes depression of activity of stretch receptors that do not carry inhibitory synapses (212), that GABA does not simulate the neural inhibition of the opener muscle of several species of crabs and crayfish (167), and that GABA depresses contraction of leg muscles of crabs by attenuating excitatory postsynaptic potentials, whilst neural inhibition appears to act later in the chain of events leading to contraction (121).

The situation with L-glutamate, and its possible role as an excitatory transmitter acting upon invertebrate muscle, is somewhat similar. L-Glutamate-sensitive sites on the abductor of the dactylopodite of the crayfish (*Cambarus clarkii*) are restricted to the regions bearing end plates (324). These regions show desensitization to continuous administration of L-glutamate, in much the same way as cholinoceptive transmitter receptor sites become desensitized in the presence of acetylcholine (334). The glutamate reversal potential is at a level near zero (324), but technical difficulties have prevented a comparison with that of the excitatory junction potential. L-Glutamate occurs abundantly in crustacean nerve (224, 327), and may be in slightly higher concentration in motor axons that in inhibitory axons (202, but see 204). L-Aspartate also occurs in crustacean nerve (224, 327), in even greater amount, but this substance has at best only a weak excitatory action on crustacean muscle (269, 357), and has even been reported to depress excitatory synaptic action (202). Moreover, it occurs equally in motor and inhibitory axons (204). The role of excitatory amino acids in nervous processes elsewhere in the invertebrate kingdom cannot be reviewed profitably at the present time; the inconsistent action of glutamate acid on snail central neurones (126; Section II B), for example, is particularly difficult to interpret. However, the sensitivity of muscles of the snail *Helix aspersa*, and of the cockroach *Periplaneta americana*, to low concentrations of L-glutamic acid, together with the presence of this amino acid in perfusates of these preparations, in amounts depending upon the number of stimuli applied to the excitatory nerves (184), strongly suggests that L-glutamic acid may be a peripheral neuromuscular transmitter in these invertebrates.

B. Vertebrate

None of the studies carried out on mammalian neurones has produced definitive evidence as to the site of action of either excitant or depressant amino

acids, but the failure of strychnine to block GABA-induced depression of motoneurones has been taken as evidence against an action of GABA on inhibitory transmitter receptor sites on these cells (69). There remains some doubt, however, that strychnine blocks inhibition by occupying postsynaptic inhibitory transmitter receptor sites, as has been generally assumed (44, 63, 64). Unfortunately, apart from cholinergic blocking agents, whose actions are limited to a minority of cells (74), no substances are known whose actions can be definitely ascribed to blockade of excitatory or inhibitory transmitter receptor sites in the mammalian central nervous system; nor, despite an extensive search involving a very large number of amino acid derivatives (80), have any agents been found which specifically block the actions of either the excitatory or inhibitory amino acids. It has therefore not yet been possible to support or exclude an action of the amino acids on transmitter receptor sites, on the basis of a similar or differential blocking action of pharmacological agents on responses evoked synaptically or by amino acids. Nevertheless, the failure of low concentrations of amino acids to affect mammalian nerve fibres (69, 230) except at terminals (73, 100, see also 290), where axo-axonic synapses occur (99), may be taken to indicate that the presence of junctional membrane is required for amino acid action in the mammalian central nervous system, just as it is for invertebrate stretch receptors and muscles. Whether such action on synaptic membrane is mediated by combination with specific transmitter receptor sites, or with some other component of the synaptic membrane (see 77) requires further discussion. In the case of the depressant amino acids in particular, it is tempting to extrapolate from the strong indications that GABA acts on inhibitory transmitter sites on the crayfish stretch receptor and muscle to the conclusion that all sites of GABA depressant action, whatever the tissue, are also inhibitory transmitter receptor sites. However, it must be remembered that GABA has a depressant action on crayfish stretch receptors which do not carry inhibitory synapses (212), and the recent observation that the action of GABA in the goldfish Mauthner cell is manifested not only on the soma, which carries a higher density of inhibitory synapses, but also on the dendrites which carry mainly excitatory synapses (88) (see Section II B) is also relevant to this issue.

GABA and like compounds exert their depressant actions on mammalian neurones by increasing membrane conductance without change of membrane potential (69, 205). These observations indicate that the conductance changes associated with amino acid depression and neural inhibitions have different equilibrium potentials, suggesting that different ionic mechanisms are involved. The GABA-induced conductance change is thought to involve mainly chloride ions (69, 77), as in invertebrates. The excitatory amino acids depolarize the membranes of mammalian motoneurones, and the reversal potential for this action is at a more polarized level than that for neural excitation (66). No detailed studies have yet been made of the ionic mechanism underlying the action of the excitatory amino acids, but it seems probable, in view of the depolarization produced, that at least sodium ions are involved (70). Because of the similarity in structure between excitant and depressant amino acids, it has been postulated

that the excitants induce the same membrane permeability change in the neurones of the mammalian central nervous system as the depressants, except that there is an additional increase in the permeability to cations in the case of the excitants (77). As the depressants probably manifest their action mainly through an increase in the membrane permeability to choride ions it would seem likely that at least sodium and chloride ions are involved in the case of the excitant amino acid action. The ionic mechanism for synaptic excitation of mammalian neurones requires further investigation (see 99), but if this be similar to that proposed for the neuromuscular junction (323), only potassium and sodium ions are involved. Such differences in ionic conductance changes would explain the different equilibrium potentials for amino acid and neural excitation.

Thus, the available evidence on the sites and mechanism of depressant and excitant amino acid action in the mammalian central nervous system does not support a transmitter role in either case. Some uncertainties do exist, however, regarding the interpretation of the above experimental observations. For instance, strychnine may act presynaptically, by blocking the release of spinal inhibitory transmitters, rather than by a postsynaptic combination with inhibitory transmitter receptor sites. If this were so, the failure of strychnine to block GABA action upon mammalian motoneurones would not constitute evidence against interaction of GABA with the inhibitory transmitter receptors of these cells. Furthermore, the postsynaptic inhibition of other mammalian central neurones is not blocked by strychnine (9, 62, 209), although the cells are readily depressed by GABA. On the other hand, the different equilibrium potentials for the actions of the depressant and excitant amino acids, as compared with those for the actions of inhibitory and excitatory transmitters, respectively, is not easily circumvented, but the possibility should not be discounted that equilibrium potentials of conductance changes induced by administering substances over a limited area of the membrane need not necessarily be identical to those measured when the same or similar substances are liberated synaptically at a large number of junctions situated fairly uniformly over the soma and dendrites (see also 324).

A great deal of circumstantial evidence has been brought to bear on the question of a possible transmitter or other neurohormonal function of the amino acids in the vertebrate central nervous system (see 272). This is related to the occurrence of the amino acids in such tissue, their distribution and those of associated enzymes in whole tissue and in subcellular fractions, and changes in such levels and distributions during maturation and after modification of normal function by a variety of means.

A detailed compilation of the levels of free amino acids in nervous tissue of a wide variety of animals has been made (327). It is hardly necessary to point out that not only GABA (15, 276, 328) and L-glutamate (328, 362) occur in mammalian central nervous tissue, but also several other amino acids which have similar depressant and excitant actions when extracellularly applied to neurones. Amongst other depressant amino acids, taurine, β-alanine, glycine and alanine (328), hypotaurine (see Fig. 5) (29) and guanidino-acetic acid (250) all occur to a

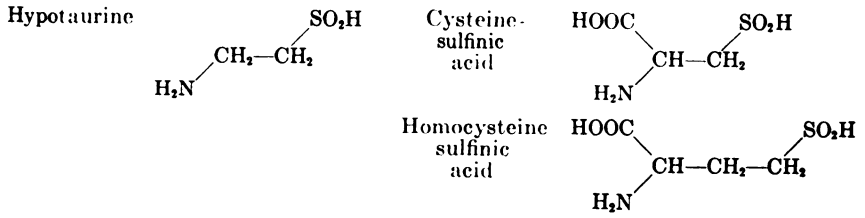

FIG. 5.

greater or lesser extent, and whilst L-aspartic acid is the only excitant amino acid other than L-glutamic acid to be found in significant quantities in central nervous tissues, the presence of at least trace quantities of cysteic acid (328) and L-cysteine sulfinic acid (see Figs. 2 and 5) (29) is to be expected. Also the strong depressant, 3-amino-1-propane sulfonic acid, and the excitants, L-homocysteic and L-homocysteine sulfinic acids (see Figs. 2 and 5), could be present in small amounts as metabolites of methionine and homocysteine, which are involved in cystathionine production (98, 329). Nevertheless, of the depressant and excitant amino acids, GABA and L-glutamate occur in largest amounts; moreover, the former occurs uniquely in nervous tissue (271, 275). Thus if any amino acids are involved in regulating nerve cell activity, as synaptic transmitters or otherwise, it seems unnecessary to look beyond these two, at least initially. In this connection it is of interest that GABA and L-glutamate are released from the cerebral cortex of the cat at a rate dependent upon its state of activation, more GABA and less L-glutamic acid being released under conditions associated with "sleep" electro-encephalographic patterns, with the reverse situation obtaining under "arousal" conditions (176). This differential release is unlikely to be connected with alterations of cortical blood flow (356) since the release of other amino acids (L-glutamine and L-aspartic acid) do not vary similarly with the different conditions. Although such experiments do not establish whether the amino acids are actually involved as transmitters, the amount collected being related to the relative activity of excitatory and inhibitory synapses, or whether the amino acid levels merely reflect the metabolic activity of neuronal tissue, it nevertheless seems significant that the other two amino acids are unaffected by the changed conditions. However, it will be necessary to extend these studies to include a greater range of amino acids and other substances.

Unlike acetylcholine, glutamic acid and GABA do not seem to be especially concentrated in nerve endings of rat brain (284, 370, 373). However, where cytoplasmic concentrations of transmitter candidates are high, one would not necessarily expect levels in nerve endings to provide evidence for or against a transmitter function. It is possibly more pertinent that glutamic acid decarboxylase, the enzyme uniquely present in high concentration in nervous tissue, and responsible for GABA formation from L-glutamate (277), is somewhat concentrated in acetylcholine-poor nerve endings, and that the enzyme seems to be bound to vesicles by calcium ions (285). It has been pointed out (285) that such a localization of GABA production would be appropriate either for inter-

mittent release as an inhibitory transmitter or (since it seems able to penetrate freely the membranes of nerve endings), for continuous release as a "regulator" of neuronal activity, although evidence has yet to be obtained that the functioning of central neurones is governed to any marked extent by such a "regulatory" mechanism. Exogenous GABA becomes bound, by a sodium-dependent mechanism, to subcellular fractions of brain which are rich in nerve endings (281, 287, 358, 371, 372).

The distribution of amino acids and related enzymes in various regions of nervous tissue has been studied (3, 4, 10, 17, 36, 154, 210, 244, 273, 280, 286, 297, 348) and regions of high rates of transamination and oxidation of GABA to succinate have been demonstrated histologically (351, 352). There has yet to be shown any correlation between the observed levels of amino acids and enzymes and the known physiological functions of the various regions (154, 352). The levels of GABA, glutamic and aspartic acids and of glutamic acid decarboxylase activity have been shown to increase during maturation (24, 31, 153, 244, 278, 279, 295, 359, 360). Of particular interest is the relation between the increase in glutamic acid decarboxylase activity and the increase in surface area of the dendrites (24), which itself can possibly be correlated with the elaboration of synaptic junctions, since glutamic acid decarboxylase is concentrated in presynaptic endings (285).

A large number of attempts has been made to correlate changes in amino acid concentrations within the whole brain, and also within specific regions, with changes in nervous activity induced by a variety of drugs and other means. A complete survey is beyond the scope of this review, but mention can be made of the effects of hydrazides (20, 162, 177, 188, 189, 190, 191, 236, 237, 340, 376), hydroxylamine (21, 22, 104), other pyridoxine antagonists and carbonyl trapping agents (23, 43, 83, 125, 181, 268, 364, 365), methionine sulfoxide and sulfoximine (198, 249, 338), pentamethylenetetrazol (125, 181, 236, 333), picrotoxin (125, 181, 236, 306, 333), insulin (58, 195, 236), psychotropic drugs (114, 245, 326; see 159), and high oxygen tension (380). Such studies have been very valuable in emphasizing the close interrelation between amino acid metabolism and the activity of the nervous system, although a close correlation of the latter with the concentrations of any one particular amino acid has not always been found (20, 181, 236, 237, 275). It is relevant that administration of the convulsant hydrazide, thiosemicarbazide, decreases the levels of GABA in brain and spinal cord (189, 275, 376), but does not alter the amplitude of inhibitory postsynaptic potentials recorded intracellularly from spinal motoneurones (332). This observation (332) can be taken as evidence against a role of GABA as a spinal inhibitory transmitter.

It is unlikely that investigations concerned with the variation of amino acid levels in whole tissue associated with induced changes in nervous activity will greatly clarify the problems involved, particularly in view of the difficulty of separating cause and effect. Greater emphasis could perhaps be given to the active uptake from suspension media of the neuropharmacologically active amino acids (112, 240, 304, 307, 308, 331, 342, 343, 344; see also 1, 6, 55, 56, 218).

Certain aspects of these active transport processes are very significant, especially the associated ion and water movements. It seems highly probable that the mechanisms of amino acid uptake in brain slices and the pharmacological actions of the same substances are associated, and the tissue molecules (receptors) which bind the amino acids may even be the same. The descending order of accumulation for a limited series of substances related to GABA is GABA, β-alanine, glycine, α,γ-diaminobutyric acid, ε-aminocaproic acid, α-amino-*n*-butyric acid, N-acetyl-GABA, GABA methyl ester (344), an order which is well correlated with the order of depressant potency in the case of cat spinal neurones (77). For glutamic acid analogues, D-glutamic acid is taken up at the same rate as L-glutamic acid, and these rates are higher than those of L-aspartic acid and D-glutamine; whilst the uptake of D-glutamic acid γ-methyl ester is very much slower than that of 4-methylglutamic acid. This order is very close to what would be predicted from structure-activity relationships for amino acid excitants of spinal and cortical neurones (60, 77). It would be interesting to test a larger series, including the more potent amino acid excitants and depressants (Section IV A, 1) to determine whether the correlation can be extended. If it could be established that the two phenomena are indeed closely related, then it would perhaps indicate that the pharmacological actions of amino acids arise as a consequence of ionic movements associated with the cellular uptake of the substances, rather than with the activation of specific inhibitory or excitatory transmitter receptors.

VI. IMPLICATIONS OF THE PHARMACOLOGICAL ACTIVITY OF AMINO ACIDS

Whether or not GABA and L-glutamic acid ultimately prove to be synaptically released transmitters in the mammalian central nervous system, or whether these amino acids are merely associated with energy metabolism, and in this way are intimately linked with the functional activity of nervous tissue (see 108), the fact that several endogenous amino acids (some of them occurring in large amounts in the mammalian central nervous system) possess significant and complementary neuropharmacological actions cannot be over-emphasized in relation to its neurological implications. There exist special mechanisms for controlling the levels of these substances in brain, such that large increases in blood levels do not result in significant increases of the levels in the cerebrospinal fluid or brain (179, 219, 274, 342, 353, 362, 374), except in immature animals (152, 362) or where the blood-brain barrier is impaired (33, 261). Nevertheless, the large number of studies carried out in recent years on the results of injected drugs and metabolic inhibitors shows that certain changes in the functional state of the nervous system are associated with, if not necessarily directly caused by, significant changes in amino acid levels in brain (see Section V).

Extracellular space represents only a small fraction of the total volume of nervous tissue, and hence the redistribution of intracellular substances need only be minimal to raise their extracellular concentrations to critical levels. For instance, the overall concentration of L-glutamic acid in brain is about 10 mM (328, 362), and the major part of this must be located intracellularly (70, 207). If the extracellular space is approximately 10% of the total, and an extracellular concen-

tration of, say 10^{-4} to 10^{-3} M is considered sufficient to depolarize neurones (70, 207), only 0.1 to 1 %, of the intracellular glutamate need be relocated extracellularly, either because of leakage from intracellular compartments, or because of impairment of uptake, to affect the activity of neurones. This figure could indeed be much lower, especially taking into account the total intracellular concentrations of all of the pharmacologically active amino acids rather than glutamate alone. These small but critical changes in extracellular levels may not necessarily bear any direct relationship to total (mainly intracellular) concentrations, and hence are unlikely to be detected by the present methods of gross analysis.

The possibility that distribution-controlling mechanisms are inhibited in certain pathological conditions, or that such malfunction is brought about by drugs, may sometimes be advantageously investigated by the use of brain slices. It may be significant, for instance, that the ability of epileptogenic cortex to accumulate amino acids is impaired (32, 35, 338, 339, 340), as is that of slices of brain tissue taken from animals subjected to high oxygen tension (182; see also 336). Certain psychotropic drugs likewise interfere with the accumulation mechanisms (114), but the convulsant pentamethylenetetrazol seems to have no such effect (252).

A proportion of the brain glutamine synthetase may be extracellularly located, since "labelled" glutamic acid applied to the rabbit cortex is converted into glutamine very rapidly without apparently first equilibrating with the bulk of the tissue glutamic acid (252). Waelsch and collaborators (30, 34, 220, 361) have likewise described the conversion of intracisternally injected glutamate into glutamine without prior equilibration with total tissue glutamate. The latter authors argue in favour of an intracellular location of the enzyme, but do not exclude the possibility that the reaction is mediated on the surfaces of cells (361). It is significant that the "compartmentalized" glutamic acid pool, which is so very rapidly converted into glutamine, may represent less than 2 % of the total tissue glutamic acid (30, 220), and could well be extracellular. Hence it seems possible that extracellular or membrane-bound glutamine synthetase may participate in the active transport of glutamic acid by conversion of the latter into its amide, which apparently can penetrate cell membranes more easily (30). This suggestion finds support in the observation that glutamine synthetase seems to be concentrated in the microsomal fraction of brain homogenates, and thus to be associated with membranous components (285). Partial explanation of the convulsant actions of the glutamine synthetase inhibitors, methionine sulfoxide and methionine sulfoximine (293, 339), may lie in raised extracellular levels of glutamic acid, despite the lowered total glutamate which could be caused by the failure of transport mechanisms and by the metabolic utilization of the intracellular supply of the amino acid. It is noteworthy that glutamine and asparagine sometimes suppress seizures in epileptic persons (338) and that these substances also correct the inability of slices of epileptogenic cortex to accumulate glutamic acid (249, 338), an effect indicative of a re-establishment of more normal transport mechanisms.

It would appear that the critical importance of amino acids in nervous function was thought likely long before there was definite experimental indications to this effect, and there have been many studies of amino acid composition of blood, urine, cerebrospinal fluid, and nervous tissue itself in attempts to find consistent patterns associated with abnormal nervous or psychic conditions (2, 196, 197, 226, 299, 363, 381). The detailed relationship between alterations of the distributions of various amino acids and neurological disorders is beyond the scope of this review, but several findings are relevant. It is possible that the seizures associated with both dietary deficiencies of pyridoxine (57, 340), and with the inhibition of pyridoxal-dependent enzymes governing GABA and glutamate levels (181, 189, 190, 376), are both related to disturbances of extracellular amino acid levels, particularly as enzyme inhibition is often accompanied by alterations of total brain GABA levels (376). The manifestation of certain inborn errors of metabolism (140) may also be linked with an effect of amino acids upon nerve cells. For example, the block of the conversion of homocysteine to cystathione, suggested as causative factor in homocystinuria (46, 50), could possibly lead to abnormally high levels of the excitant, homocysteic acid. It may be relevant that cystathione levels are also low in epileptic brain tissue (244, 381). The possible link between β-N-oxalyl-L-α,β-diaminopropionic acid and lathyrism has been mentioned (Section II A, 2) but it should be pointed out that many other toxic substances have been suggested for this disorder (26, 222, 266, 289, 294). The central depressant action of both γ-hydroxybutyric acid (215) and γ-butyrylactone (129) may also be due to an interference with cerebral GABA distribution, although neither substance produces a change in the overall GABA levels (129, 215, 283).

Glutamic acid, glutamine, asparagine and GABA (337, 338, 360, 362, 369) have been used in the treatment of epilepsy, with somewhat inconsistent results. Glutamic acid was for a time also hailed as an intelligence-increasing agent and fed in large quantities to the mentally deficient (360, 362, 369). Although the efficacy of the treatment is debatable, and nowadays it is known to be impractical to try to increase brain or cerebrospinal fluid levels of glutamate by such means, it would nevertheless seem worthwhile to re-examine the problem from new angles. For instance, there are indications that glutamic acid is clinically effective only where there are present secondary deficiencies, which may impair the functioning of the blood-brain barrier (362). The recent finding that aminooxyacetic acid lowers the barrier of mice and rabbits to intravenously administered GABA, causing sedation (352), suggests that greater clinical effectiveness of glutamate (or GABA) may likewise follow the simultaneous administration of agents which similarly affect the barrier system. An alternative line of attack would be the development of inactive amino acid derivatives which could pass the blood-brain barrier and react with extracellular enzymes to release the neuropharmacologically active substances (see 51). The uptake of the substances into neurones and de-activation by a variety of means (*e.g.*, glutamate to glutamine, aspartate to N-acetylaspartate, GABA to succinic semialdehyde) may be in some cases desirable, and where not, there exists a large number of

synthetic amino acids (54, 78, 79, 366) whose specific permeability properties or lack of enzymic inactivation, or both, may prove useful.

Note Added in Proof

Since the completion of this review, it has been reported that electrophoretically administered GABA hyperpolarizes feline Deiter's neurones (385). These cells are inhibited monosynaptically by impulses in Purkinje cell axons (384), and such inhibitory potentials are increased in amplitude by hydroxylamine (383). When taken in conjunction with the comparatively high GABA content of Purkinje cells (383, see also 351), these results strongly suggest that GABA is indeed the inhibitory transmitter released at Purkinje cell axonal terminals. However, full acceptance of this possibility awaits a comparison of the equilibrium potentials for the inhibitory and GABA-induced hyperpolarizations, and an explanation of the failure of hydroxylamine to alter the time course of the inhibitory potential (383).

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