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## Mechanisms of Inhibition of Cerebellar Purkinje Cells in Rat and Frog

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Purkinje cells respond to iontophoresis of norepinephrine (NE) by reduction of spontaneous discharge rate. Cyclic AMP (C-AMP) also depresses rate and a large body of other pharmacological evidence supports C-AMP as the postsynaptic mediator of NE effects. When rat Purkinje cells are recorded intracellularly during extracellular drug applications, NE and cyclic adenosine nucleotides hyperpolarize, without decreasing membrane resistance, distinguishing it from classical inhibition. NE-containing nerve terminals synapse with Purkinje cells, as shown by light and electron microscopic histochemistry. In attempts to activate this pathway selectively, several NE-containing brain stem nuclei were discretely stimulated with bipolar concentric electrodes. Positive inhibitory effects were obtained only with locus coeruleus (LC) stimuli: trains of 10–150 pulses at 5–20/sec produced dramatic cessation of firing of Purkinje cells, lasting 12–65 sec, but not of interneurons. Single LC stimuli produce inhibition of 60–400 msec. Cerebellar NE axons and LC inhibitory effects are eliminated by chronic pretreatment with 6-hydroxydopamine, or by acute treatment with reserpine and alpha methyl tyrosine. Purkinje cells also respond to gamma amino butyric acid (GABA) by reducing discharge rate. This amino acid hyperpolarizes Purkinje cells while decreasing membrane resistance, analogous to classical inhibitory postsynaptic potentials. In frogs, inhibition of Purkinje cells by molecular layer interneurons is blocked by the iontophoretic administration of the GABA antagonists picrotoxin or bicuculline, but not the glycine antagonist, strychnine. These studies provide evidence for two types of inhibitory pathways to Purkinje cells: a unique inhibitory pathway from locus coeruleus mediated by NE, and a more conventional cortical interneuronal inhibitory pathway mediated by GABA.

## Mechanism of Action of Psychotomimetic Drugs in the Brain Stem

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Recent investigations with three derivatives of lysergic acid have shown that their potencies as antagonists to the excitatory effects of 5-HT closely paralleled their psychotomimetic potencies, LSD 25 being the most potent and BOL 148 the weakest[1].

It has been suggested[2] that methylated amines, arising from abnormalities in methylation or demethylation reactions in the CNS, may be involved in the aetiology of schizophrenia. Since several methylated tryptamine derivatives, which are present in relatively large amounts in the blood of schizophrenics[3] are known to possess psychotomimetic activity similar to that of LSD 25[4,5,6,7], the actions of several tryptamine derivatives, and their interactions with 5-HT and other putative transmitters have been studied using the iontophoretic technique. The effects of these substances are complex: on some neurones their effects are similar to those of 5-HT but with different time courses; on others the effects of 5-HT were specifically antagonized. Results will also be presented from studies in animals pretreated with reserpine or PCPA in order to deplete 5-HT in the brain.

1 R. J. Boakes, P. B. Bradley, I. Briggs and A. Dray, Br. J. Pharmac. *40*, 202 (1970).
2 H. Osmond and J. R. Smythies, J. ment. Sci. *98*, 309 (1952).
3 B. Heller, N. Narasimhachari, J. Spaide, L. Haskovec and H. E. Himwich, Experientia *26*, 503 (1970).
4 F. Benington, R. D. Morin and L. C. Clark, Alabama J. med. Sci. *2*, 397 (1965).
5 Z. Böszöményi, P. Dér and T. Nagy, J. ment. Sci. *105*, 171 (1959).
6 S. Szara, in: *Psychotropic Drugs* (Eds. S. Garattini and V. Ghetti; Elsevier, Amsterdam 1957), p. 460.
7 A. T. Shulgin, in: *Psychotomimetic Drugs* (Ed. D. H. Efron; Raven Press, New York 1970), p. 119.

## Glycine and GABA Actions in Hypoglossus Nucleus and Blocking Effects of Strychnine and Picrotoxin

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Effects of neutral ω-amino acids on hypoglossus motoneurones were studied with intracellular recording and extracellular analysis of antidromic field potentials in cats and rabbits. Drugs were applied iontophoretically with cationic currents (glycine and GABA 0.5 *M*, adjusted with 0.1 eq. HCl; strychnine as 10 m*M*, picrotoxin as saturated solution in 165 m*M* NaCl) from 3- or 4-barrel pipettes glued parallel to a single recording electrode.

On hypoglossus motoneurones, neutral ω-amino acids had principally the same actions as described in spinal cord motoneurones[1,2], including hyperpolarization and conductance increase of the membrane. A comparison of the relative strength of glycine and GABA actions was made on the prominent antidromic field potential[3]. In 70% of the electrodes tested, glycine clearly was more effective than GABA (comparing currents of similar magnitude), in 12% GABA was the more effective substance, and in 18% no distinct difference was detected. Glycine actions were antagonized selectively by strychnine (also when intravenously applied), while picrotoxin clearly blocked GABA actions. Picrotoxin affected glycine actions slightly only with high currents (> 120 nA).

The results show that hypoglossus motoneurones, located in the brain stem, behave in principle similarly to spinal cord motoneurones in respect to the actions of neutral ω-amino acids. The rather selective blocking effect of picrotoxin against GABA, reported previously in Deiters[4,5] and oculomotor[6] nuclei, is also confirmed.

1 D. R. Curtis, L. Hösli, G. A. R. Johnston and J. H. Johnston, Expl. Brain Res. *5*, 235 (1968).
2 R. Werman, R. A. Davidoff and H. M. Aprison, J. Neurophysiol. *31*, 81 (1968).
3 R. Lorente de Nó, J. cell. comp. Physiol. *29*, 207 (1947).
4 G. ten Bruggencate and I. Engberg, Brain Res. *25*, 431 (1971).
5 K. Obata, K. Takeda and H. Shinozaki, Expl. Brain Res. *11*, 327 (1970).
6 K. Obata and S. M. Highstein, Brain Res. *18*, 538 (1970).

## Amino Acid Antagonists

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Useful information regarding the nature of the transmitter at a particular set of synapses may be provided by the use of a specific antagonist: a compound which blocks synaptic transmission at postsynaptic sites should also block the action of a substance suspected of being the transmitter. There are, however, considerable difficulties in comparing the effects of electrophoretically and systemically administered agents on both synaptic processes and inhibition or excitation induced by the artificial administration of a putative transmitter near neurones.

The observation that strychnine, and a number of related substances which block certain types of spinal inhibition, are all glycine antagonists provides support for proposals that glycine is of considerable significance as an inhibitory transmitter in the spinal cord. On the other hand, bicuculline, which does not modify these strychnine-sensitive inhibitions, does reduce the prolonged inhibition of spinal reflexes which are sensitive to picrotoxin, and also influences synaptic inhibitions in the cerebral cortex, cerebellum, hippocampus, ventrobasal thalamus, lateral vestibular nucleus and olfactory bulb. Since bicuculline is a selective antagonist of the inhibitory effect of GABA upon neurones in these various regions of the CNS, these observations support other types of evidence that GABA is of major significance as an inhibitory transmitter operating throughout the brain and spinal cord.

Specific antagonists are being sought in order to establish transmitter roles for excitant amino acids.

## Release Processes for Neurohormones and the Secretion of Neurotransmitters

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The release of hormones from the neurohypophysis and the release of neurotransmitters at chemical synapses both depend on the entry of calcium into the nerve terminals following their depolarization by invading action potentials. Isolated rat neurohypophyses were studied in vitro and the octapeptides released on electrical stimulation of the pituitary stalk or on exposure to excess potassium were estimated by a milk-ejection assay. The addition of tetrodotoxin (TTX) to the incubation medium abolished the compound action potential recorded from the neural lobe following electrical stimulation, as well as the stimulus-evoked hormone release. Resting release, however, was unaffected by TTX. In TTX-treated neurohypophyses, excess potassium was still effective in eliciting graded secretory responses, thus indicating the independance of the release process from the increase in sodium conductance responsible for the propagation of action potentials[1].

The presence of external sodium is not an essential requirement for hormone release; in fact, in Na-free Locke solutions, electrical stimulation of the pituitary stalk still evoked an enhanced output of neurohypophysial hormones. In media in which the concentrations of $Na^+$ and $Ca^{++}$ were varied, release depended on the $\frac{Ca^{++}}{(Na^+)^2}$ ratio, an observation that indicates competitive antagonism between Na and Ca ions at the level of release triggering sites.

These and other parallels support the concept[2] that the release processes for neurohormones and for neurotransmitters may be essentially similar.

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[1] J. J. Dreifuss, I. Kalnins, J. S. Kelly and K. B. Ruf, J. Physiol., Lond. *215*, 805 (1971).
[2] W. W. Douglas, Br. J. Pharmac. *34*, 451 (1968).

## Facilitation at the Crayfish Neuromuscular Junction, the Effect of Hyperpolarization of the Terminals and of Ouabain

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In terminal regions of crayfish motor fibres potential changes generated in the terminal and postsynaptic potentials were recorded using extracellular microelectrodes. At 100–200 μm distance from the recording site a nerve branch was cut and sucked into a capillary electrode. Insulation at the electrode tip either was established by close fit of the capillary[1] or by a sucrose gap within the suction electrode. Through this electrode polarizing current could be supplied and action potentials of the nerve fibre could be measured.

During application of hyperpolarizing current the amplitude of the postsynaptic potentials increased by factors of 1.3–4.1. This was due to an increased probability of release of quanta, the effects of hyperpolarization thus were analogous to synaptic facilitation[2]. Also similar to states of synaptic facilitation the frequency of spontaneous postsynaptic potentials decreased during hyperpolarization. The potentials recorded from the nerve terminal were increased and prolonged during hyperpolarization, and the action potentials recorded from the motor nerve fibre through the sucrose gap suction electrode increased from 15 to 22 mV during the application of $-0.1$ μA of hyperpolarizing current, while the duration of the action potential was prolonged from 4 to 9 ms. Similar effects on the nerve terminal potentials also were seen during synaptic facilitation[3].

These results suggested that hyperpolarization of the terminal might be the basis of synaptic facilitation. One possible mechanism of hyperpolarization might be the activation of an electrogenic $Na^+$-pump during repetitive stimulation. However, application of $10^{-4}M$ ouabain for 1 h did not decrease facilitation.

[1] J. Dudel, Pflügers Arch. ges. Physiol. *324*, 227 (1971).
[2] J. Dudel and S. W. Kuffler, J. Physiol. *155*, 530 (1961).
[3] J. Dudel, Pflügers Arch. ges. Physiol. *282*, 323 (1965).

## Acetylcholine Turnover Investigated at a Subcellular Level in the Electric Organ of Torpedo

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Acetylcholine (ACh), the transmitter of the nerve-electroplaque junction, is stored in nerve terminals in

two compartments. The 'bound ACh' represents 60% of the total ACh; it is associated to synaptic vesicles which can be purified directly from a homogenate of electric organ[1]. The 'free ACh' is the fraction destroyed during homogenisation unless cholinesterase has been inactivated.

Electrophysiological, biochemical and morphological controls show that fragments of electric organs remain undamaged after several hours of incubation in a appropriate saline medium. A brief train of stimuli applied to the nerve terminals is sufficient to convert an important amount of ACh into choline; recovery occurs at a slow rate. During stimulation, the 'free ACh' dramatically decreases, but the amount of 'bound ACh' remains fairly constant even when the electrical response is abolished. $^{14}$C-choline added to the medium is incorporated into both ACh compartments. During series of successive periodes of stimulation, the specific radioactivity (SA) of the 'bound ACh' is little or not changed; in contrast the SA of the 'free ACh' is markedly modified indicating resynthesis. The SA of the released transmitter shows changes parallel to those of the 'free ACh' and is very different from the SA of the 'bound ACh'. The actual nature and localization of the 'free ACh' has not been elucidated. The newly synthetized transmitter should be extravesicular since choline acetyltransferase is not associated to synaptic vesicles[1]. It is concluded that the 'free ACh' is directly involved in both the synthesis and release of transmitter and that the 'bound ACh', i.e. the vesicular ACh, behaves as a stationary compartment.

[1] M. Israël, J. Gautron and B. Lesbats, J. Neurochem. *17*, 1441 (1970).

## Problems of Synaptogenesis in the Cerebellum

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The development both of the neurones and of their synaptic connections in the cerebellum raises many fundamental problems in neurobiology. For example a differentiating mitosis of the same stem cells of the external granular layer gives rise to neuroblasts that develop into either the excitatory granule cells or the inhibitory basket and outer stellate cells. Evidently at this mitosis there is established a genetic specification for production of excitatory transmitter on the one hand and inhibitory transmitter on the other. It is probable that a comparable differentiating mitosis deeper in the developing cerebellum gives rise to the excitatory nuclear cells and the inhibitory Purkyně cells and Golgi cells. In order to meet their synaptic donors, the incoming mossy fibers, granule cells migrate downwards from the molecular layer to form the granular layer, but leave their axons as the parallel fibers of the molecular layer. Similarly, in order to meet their synaptic donors, the Purkyně cells migrate upwards to the molecular layer, leaving their axon terminals to make synapses on the nuclear cells. It appears in this way that much of the regional specificity of connections derives from the original loci of the neurogenesis. Neurones are endowed with receptors for both kinds of inhibitory transmitter, GABA and glycine, though usually only one type of transmitter site attracts inhibitory synapses. It is therefore suggested that the specificity of synaptic connections is established by axon terminals actively seeking appropriate sites for the action of their transmitter substances. However it is probable that more subtle surface recognition is also operative in guiding synaptogenesis.

## On the Actions of Noradrenaline in Spinal Cord Motoneurones

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Microelectrophoretic applications of noradrenaline (NA) to certain types of neurones have been shown to hyperpolarize their cell membranes by a mechanism that differs from that of the inhibitory amino acids. In motoneurones the NA hyperpolarization causes an increase in the amplitudes of action potentials, when their generation is not completely inhibited, and often a slight increase in the amplitudes of EPSPs; IPSPs are normally decreased. Intracellular injection of chloride ions or passage of a hyperpolarizing current adequate to reverse IPSPs does not reverse the NA hyperpolarization. Reversed IPSPs increase in amplitude when NA is applied. In cells, depolarized to the level where the antidromic spike invasion becomes blocked, NA reversibly restores the soma-dendritic spike.

Measurements of membrane conductance by the injection of current pulses via the recording intracellular electrode do not show any conductance increase. Neither do they show a conductance decrease of the magnitude that has been reported from investigations of NA actions on cerebellar Purkinje cells. In this respect the mechanism of the NA hyperpolarization of motoneurones is similar to that of sympathetic ganglion cells and may involve active ion transport. However, we have found a small conductance decrease which, if due to a change in sodium permeability, could explain the hyperpolarization. In measurements of such small conductance changes, particular care must be taken to avoid influences from variations in electrode resistance.

The actions of NA are enhanced by protriptyline. This observation could reflect a blocking of NA uptake by the monoaminergic nerve terminals that have been shown to exist in the vicinity of motoneurones.

## Monoamines and the Excitatory Nigro-Striatal Synaptic Linkage

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An identified population of caudate neurones has been demonstrated to respond monosynaptically to stimulation of the substantia nigra (SN). Under a variety of anaesthetic conditions, dopamine, noradrenaline and serotonin injected microiontophoretically have failed to induce any change in this synaptic excitability. When these cells are made to fire by glutamate or aspartate, the discharge can be depressed by SN stimulation subthreshold for excitation. However, this inhibitory effect of nigral stimulation is unable to block the nigral excitatory input. Such inhibitory effects may explain the regular and long lasting increase in the latency of responses observed after prolonged tetanic stimulation at $> 25$ Hz[1] and after microiontophoretic injections of glutamate. Furthermore the amino-acid induced firing proved to be depressed by monoamines and possibly by acetylcholine. The specificity of the inhibitory effect is therefore questionable and it may involve more than one neighbouring neurone. In conclusion, most of the caudate cells excited by a direct nigro-caudate pathway, composed of fine fibres (mean latency of response for

278 cells: 18.2 ± 5.8 msec, S.D.) cannot be shown to receive an excitatory synaptic dopaminergic input, on the basis of iontophoretic studies. The pharmacology of nigro-caudate inhibitory effects mimicked by dopamine when caudate neurones are excited by amino-acids, seems to call for further electrophysiological analysis of the specificity of nigral stimulation and of the intrinsic organization of the caudate nucleus.

P. FELTZ and J. S. MACKENZIE, Brain Res. *13*, 612 (1969).

## Excitatory Actions of GABA and of Inhibitory Neurons

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Gamma-aminobutyric acid (GABA) like inhibitory neurons causes a conspicuous increase of $Cl^-$-conductance at subsynaptic and chemically excitable membrane areas of many types of nerve and muscle cells. Since the $Cl^-$-equilibrium potential is usually close to the resting potential, the effect is that of inhibition of impulse generation. Provided the resting membrane shows a low permeability to $Cl^-$, it is possible to temporarily reduce the external $Cl^-$-concentration without causing a significant depolarization. By electrotonic spread to neighboring membrane regions which are not themselves affected by the transmitter (GABA) these regions can become excited and the effect can sum with that of epsp's. In stretch receptor neurons of crayfish the frequency of spike generation is linearly and inversely proportional to the membrane potential. Electrophoretic injection of $Cl^-$ in the neuron, or reduction of the external $Cl^-$-concentration cause ipsp's and GABA to have a depolarizing action resulting in an increase in the frequency of firing. In crustacean muscle conducted action potentials are the exception; contraction is elicited by epsp's and depolarization. In muscle fibers with low $Cl^-$-permeability it is possible to produce powerful contraction by applying GABA in a medium of low $Cl^-$-concentration. It is very possible that normally occurring changes in intracellular $Cl^-$-concentration modify the response of postsynaptic cells to the transmitter released from inhibitory neurons and that such a mechanism plays an important role in changing the performance of neuronal circuits, and thus in animal behavior.

## Induction of RNA Synthesis by Stimulation of the Postsynaptic Membrane in a Mammalian Sympathetic Ganglion

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Modifications of the RNA synthesis induced by neuronal stimulation were studied in the sympathetic ganglion of the rat in order to investigate the mechanisms which enable the neuronal activity to modify RNA synthesis. The effect of neuronal stimulation was determined by measuring the variations of the specific radioactivity of RNA extracted from ganglia incubated in a modified Eagle's medium and labelled by tritiated uridine.

Preganglionic stimulation caused temporarily a reduction of the incorporation of the precursor followed by an increase. Electrophoresis of RNA performed on 2–6% exponential gradient polyacrylamide gels (MIRAULT and SCHERRER, submitted to Eur. J. Biochem.) showed that the increase in RNA specific radioactivity was essentially confined to heavy RNA. Neuronal stimulation during the blockade of the synaptic transmission by *d*-tubocurarine or mecamine had no measurable effect on the incorporation of tritiated uridine. Externally applied acetylcholine (ACh) induced changes in the RNA specific radioactivity that were similar to those observed during the preganglionic stimulation. Preventing the generation of the action potential by tetrodotoxin (TTX) or depolarizing the neurons by KCl did not change the effect of ACh on the specific radioactivity. Controls indicated that neither TTX nor depolarization affected the precursor incorporation. These results suggest that neuronal stimulation by endogenous or exogenous ACh increases the RNA synthesis and that the signal inducing the observed metabolic changes arises from the activated postsynaptic membrane.

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## Effects of Serotonin (5-HT) and some Related Indole Compounds in a Mammalian Sympathetic Ganglion

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The literature on the actions of 5-HT in autonomic ganglia is controversial, some authors having observed only an excitatory effect while only inhibition was reported by other investigators.

This study was carried out on the cat superior cervical ganglion in situ. Ganglionic surface potentials and the electrical activity in the postganglionic externalcarotid nerve were recorded in response to injections of 5-HT and related compounds into the carotid artery.

The most prominent effect of 5-HT was a rather longlasting, dose-dependent depression of ganglionic transmission. This depression was accompanied by and related in its time course to a ganglionic hyperpolarization, to a reduction of the postpositivity and an increase of the postnegativity of the ganglionic action potential. The threshold dose for synaptic inhibition was extremely low – on the average $10^{-11}$ mole (about 1.7 ng of 5-HT base). The inhibitory effect increased slowly over a dose range of 5 log units, however, responses to single supramaximal preganglionic stimuli could not be abolished. At higher doses a small ganglionic depolarization of only several seconds duration accompanied by asynchronous discharges and facilitation of synaptic transmission occurs. 5-HT-induced stimulation reached its maximum already with doses 10 to 30 times threshold; the sensitivity of ganglia to the excitatory action was increased by prior tetanic stimulation. Depolarization and facilitation was followed by depression and two phases of hyperpolarization. An immediate shortlasting hyperpolarization resembled that observed after depolarization by nicotinic agents, the longlasting hyperpolarization was obviously the effect already observed with doses subthreshold for stimulation. Bufotenin had qualitatively identical actions, whereas lysergic acid diethylamide and psilocybin showed only the inhibitory effect of 5-HT. α-Methyl-5-HT had no inhibitory actions and its stimulating effect was blocked by hexamethonium.

## Glutamate as a Factor in the Mechanism for Fluid Shifts in Central Nervous Tissue

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There is evidence that during asphyxiation and spreading depression extracellular material is transported into the intracellular compartment as shown by an increase in tissue impedance, an accumulation of chloride in cellular elements and a loss of extracellular space in electron micrographs of freeze substituted material. This transport has been ascribed to an increase in Na permeability of plasma membranes caused by a release of glutamate from the intracellular compartment. The latter concept is supported by a release of labelled glutamate from isolated chicken retinas charged with $^{14}C$ glutamate, during stimulation with direct current or solutions of KCl or unlabelled glutamate, which elicit spreading depression in the cerebral cortex.

Spreading depression in the retina causes an increase of the tissue transparency due to the uptake of extracellular material by the intracellular compartment and thus by an increase in Na permeability. The glutamate release and the transparency change are affected differently by continued application of glutamate in low concentrations which causes desensitization of the transparency change elicited by stimulating glutamate concentrations, but not of the glutamate release. Magnesium ions suppress the transparency change but not the release of the label caused by application of the unlabelled amino acid. Homocysteic acid causes the transparency change without a glutamate release. These observations could be explained by the presence of independent Na and glutamate channels either in the plasma membrane of the same or of different structures. Both channels would be affected by glutamate. Desensitization, Mg ions and homocysteic acid would affect the Na, but not the glutamate channels.

## Effects of Glycine on Bulbar Reticular Neurones in vivo and Spinal Neurones in Tissue Culture

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It has been shown that glycine is a more potent depressant of bulbar reticular neurones than GABA, and that strychnine reversibly blocks the depressant action of glycine but not that of GABA[1]. In contrast bicuculline often reversibly reduced the depression caused by GABA but only sometimes affected the depression by glycine[2].

The action of glycine on the membrane potential and the membrane conductance was studied using a single KCl-electrode for intracellular recording and a 4-barrel micropipette for extracellular drug application. The depression of reticular neurones by glycine was accompanied by a hyperpolarization and an increase in membrane conductance. The hyperpolarization was sometimes reversed to a depolarization presumably due to the spontaneous diffusion of chloride ions from the recording electrode. These observations provide some evidence that glycine may be the major inhibitory transmitter in the medulla oblongata, as has been proposed for the spinal cord[3,4].

Glycine also caused a hyperpolarization and an increase in membrane conductance of large spinal neurones (probably motoneurones) which have been grown for 13–26 days in tissue cultures (method for cultivation see[5]). These results suggest that spinal neurones grown in vitro have similar pharmacological properties as those observed in vivo[3,4].

[1] L. Hösli and A. K. Tebēcis, Expl Brain Res. *11*, 111 (1970).
[2] A. K. Tebēcis, L. Hösli and H. L. Haas, Experientia *27*, 548 (1971).
[3] D. R. Curtis, L. Hösli, G. A. R. Johnston and I. H. Johnston, Expl Brain Res. *5*, 235 (1968).
[4] R. Werman, R. A. Davidoff and M. H. Aprison, J. Neurophysiol. *31*, 81 (1968).
[5] E. Hösli and L. Hösli, Brain Res. *30*, 193 (1971).
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## The Present Status of the Vesicle Hypothesis

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The finding of vesicles in nerve terminals at synapses and the finding that acetylcholine (ACh) release, both spontaneous and in response to nerve impulses, always occurred in uniform, multimolecular packets led to the hypothesis that the vesicles contained and released the packets of ACh. Further electron microscopic and electrophysiological observations have shown the ubiquity of presynaptic vesicles and quantal release at chemically transmitting synapses. More recently vesicles from known synapses have been isolated and shown to contain not only the transmitter but proteins and in cases of adrenergic vesicles, ATP. Furthermore, protein and transmitter can be found together in perfusates from stimulated preparations. These investigations are complemented by studies correlating changes in vesicle number, volume and distribution as seen in electron microscopy with perturbations of release, particularly of quantal size.

The major remaining problems are the nature of the interaction between vesicle and nerve terminal membrane and the mechanism by which a vesicle releases its contents, the so-called reverse pinocytosis or exocytosis. To account for the life span of vesicles and transmitter it is necessary to assume continued refilling and reemptying of vesicles. It seems possible that upon emptying the vesicles are partly refilled with extracellular material which, after reformation of the vesicle, is removed. The granular core of some vesicles indicates that they contain catecholamines. Research would be much speeded if it were possible to distinguish either full or empty vesicles at cholinergic junctions.

## Synaptic Transmission Mechanisms in the Cerebellar and Vestibular Inhibition

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In cats and rabbits, transmission mechanism of inhibitory synapses were investigated at three loci; 1. synapses supplied by Purkinje cells to Deiters neurones; 2. those

supplied by superior vestibular nucleus neurones to IIIrd nucleus neurones; 3. those supplied by medial vestibular nucleus neurones to $C_1$ spinal neurones. With the inhibitory postsynaptic potentials produced via these synapses, the unitary composition, temporal facilitation and depression, and frequency potentiation characteristics were analysed and compared with those in excitatory synapses. The inhibitory action of Purkinje cells of flocculus was indicated by a reflexological technique; the secondary vestibular volley evoked in IIIrd nucleus by stimulation of VIIIth nerve was depressed very effectively after stimulation of flocculus. This provided a very good material for further studying physiological and pharmacological properties of Purkinje cell inhibition.

### Autoradiographic Studies of the Distribution of $^3$H-GABA in Mammalian CNS

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The distribution of uptake sites for $^3$H-GABA in rat brain and spinal cord slices and homogenates has been studied by autoradiographic techniques at the light microscope and electron microscope levels. Preliminary studies established that radioactivity accumulated by such preparations could be entirely accounted for as unchanged $^3$H-GABA, and that a major proportion of this radioactivity could be retained in the tissues after glutaraldehyde fixation.

In slices of cerebral cortex, electron microscope autoradiography indicated a specific localization of $^3$H-GABA over nerve terminals and preterminal axons, which accounted for 82% of all autoradiographic activity. Not all nerve terminals became labelled after exposure to $^3$H-GABA; only about 30% of all terminals showed autoradiographic activity. In homogenates of cerebral cortex and other regions of the rat CNS labelling was again confined to nerve terminals, and the proportion of labelled terminals varied from 13% in cerebellum to 42% in hippocampus homogenates.

When the surface of the cerebral cortex was exposed to $^3$H-GABA in vivo, light microscope autoradiography revealed intense labelling of the superficial neuropil, and also heavy labelling of a small number of neurone cell bodies in the superficial cell layers of the tissue. It is suggested that the autoradiographic technique offers a unique opportunity for identifying the cellular elements in the mammalian brain which contain and take up GABA. The results obtained so far suggest that such sites are restricted to a specific population of nerve cells and their terminals, and suggest that GABA may be involved as a transmitter at a high proportion of all synapses in the mammalian CNS.

### Synaptic Organization of an Invertebrate Ganglion

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Electroanatomical techniques can be used in invertebrates to study synaptic interconnections on a cell to cell basis. These cellular mapping techniques have now been used in two ways: 1. to examine the principles that determine the functional expression of the interconnection between neurons and 2. to relate neuronal and synaptic properties to behavior and its modification. Based upon studies in *Aplysia*, examples of each of these two approaches will be considered.

### The Effects on Inhibition of AOAA Evoked Increase in the Cortical GABA Level

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The role of $\gamma$-aminobutyric acid (GABA) as the inhibitory transmitter in the cerebral cortex is based on the demonstration that 1. GABA is present, 2. the permeability change caused by iontophoretic applications of GABA to the postsynaptic membrane is indistinguishable from that caused by the endogenous transmitter, 3. GABA is released from the cortex during repetitive surface stimulation. The collection of GABA from the surface of the cortex is facilitated by pretreatment of the animals with amino-oxyacetic acid (AOAA) to inhibit GABA-$\alpha$-ketoglutaric transaminase. Inhibition of the enzyme however is associated with a 4- to 5-fold rise in the brain GABA level. After the intraperitoneal injection of AOAA (20–40 mg/kg), in cats anaesthetized with sodium pentobarbitone, the frequency of the glutamate evoked background discharge of single cortical neurones can only be maintained at the pretreatment level (60 min control period) by increasing the release of glutamic acid several fold. The inhibitory response to single shocks applied to either the surface of the cortex or the pyramidal tract, determined from poststimulus latency histograms, is increased. When the AOAA dosage was 20 mg/kg, recovery occurred within approximately 2 h at a time when the GABA level was still elevated in control experiments. The increase in duration of the synaptically mediated inhibition may be due to an embarrassment of the GABA inactivation mechanisms, since the inhibitory response to iontophoretic GABA applied as 10–30 msec pulses is also potentiated. The results would support the view that GABA collected from the surface of the cortex has a synaptic origin.

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### The Effect of Strychnine on Acetylcholine and Dopamine Receptors in Aplysia Neurons

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In neurons in the visceral ganglion of *Aplysia*, the addition of strychnine (STRY) in concentrations of $10^{-5}$ to $10^{-3}M$ to the bathing medium results in a reduction and/or blockage of most cholinergic and noncholinergic PSPs as well as direct depressive effects on the neuron soma and axon. In order to determine whether the effect on PSPs is due to a pre- or postsynaptic action, we investigated the effects of STRY on depolarizing (D-) and hyperpolarizing (H-) responses to acetylcholine (ACh) and dopamine (DOP) of identifiable neurons.

Sodium-dependent ACh-D-responses were reduced by a curare-like competitive antagonism, as revealed by a parallel shift to the right of the dose-response-curves

(DRCs) of about 1 log unit per 10-fold increase in STRY concentration. Similar shifts in the DRCs were found for DOP-D-responses, which were most easily blocked by STRY, for most chloride-dependent ACh-H-responses, and for DOP-H-responses. The latter were the least reduced, correlating with the minimal effect of STRY on ILDs. Therefore, STRY appears to react directly with at least four different receptors. The exception to the antagonistic action of STRY was the late component of the ACh-H-response of cells of the L2–L6 group which was increased. In these cells STRY also enhanced the late cholinergic IPSPs produced by intracellular stimulation of interneuron L10 and slightly reduced the early cholinergic IPSPs. These agonistic effects of STRY were enhanced by increasing calcium concentration, were mimicked by hexamethonium, and were probably not due to an anticholinesterase action since they occurred with carbachol responses also and showed complex interactions with eserine.

## Frequency Characteristics of the Excitatory and Inhibitory Synaptic Action of Propriospinal Neurones

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An exact comparison of the activity characteristics of central excitatory and inhibitory synapses is usually difficult because we cannot directly stimulate the corresponding neurones. But there is a structure in the spinal cord which allows such direct stimulation and the recording of the postsynaptic potentials produced by it. This structure is formed by axons of the propriospinal neurones in the dorsolateral funicle which can be stimulated separately from the long fibers in the funicle when they are degenerated. Degeneration was caused by preliminary ipsilateral hemisections of the spinal cord 10–14 days before the main experiment.

A direct stimulation of the dorsolateral funicle in such conditions produces via excitation of propriospinal axons *monosynaptic* EPSPs predominantly in flexor motoneurones and *monosynaptic* IPSP – in extensor motoneurones of lumbar segments. Both monosynaptic influences extend over 5 segments; they can be followed by more complicated polysynaptic effects. Both the excitatory and inhibitory propriospinal monosynaptic actions are effectively potentiated even during low-frequency rhythmic stimulation (10–20 stim./sec) showing identical frequency-dependance but completely different from that of primary afferent monosynaptic EPSP and propriospinal polysynaptic PSP in the same motoneurones.

A conclusion is made that the inhibitory synaptic action in the spinal cord can be mediated not only through specialized short-axon inhibitory neurones, but also through neurones with relatively long axons which are probably similar to excitatory neurones except the kind of transmitter liberated.

## Correlation between Nerve Terminal Size and Transmitter Release

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Attempts were made to determine whether the number of quanta (packets) of transmitter released by a nerve impulse is related to the size of the presynaptic terminal at neuromuscular junctions and at central synapses. End-plate potentials were recorded intracellularly at the frog neuromuscular junction. The muscle was subsequently subjected to 'cholinesterase staining', and the area of the individual end-plates studied with intracellular electrodes was measured. It was assumed that the area of end-plates so stained is proportional to the size of the motor nerve terminals. The mean number of quanta released by a nerve impulse (mean quantum content) was found to be positively correlated with the size of motor nerve terminals. There was also a positive correlation between terminal size and the frequency of spontaneous miniature end-plate potentials. The analysis was extended to synapses in the spinal cord of the cat. Monosynaptic EPSPs were recorded intracellularly from motoneurons and dorsal spinocerebellar tract (DSCT) neurons following stimulation of single afferent fibers. The mean quantum content measured was about six times greater in DSCT neurons than in motoneurons. Electron-microscopic studies showed that presynaptic terminals on motoneurons are less than 4 μm in diameter, whereas a substantial number of terminals on DSCT neurons are larger than 5 μm, ranging up to 15 μm. It is suggested that the number of quanta of transmitter released by a nerve impulse depends on the size of the presynaptic terminal.

## Studies on the Ionic Conditions of Hyperpolarizing Postsynaptic Inhibition

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Reversal potentials ($E_{IPSP}$) of inhibitory postsynaptic potentials in cat spinal motoneurons shift towards resting membrane potentials ($E_M$) after systemic and extracellular electrophoretic application of $NH_4^+$-ions in an estimated concentration range of 1 to 3 m*M*/kg. The slow time course of the action of $NH_4^+$ suggests an indirect, not diffusion controlled mechanism[1]. Passive membrane properties remain essentially unaltered. During the action of $NH_4^+$ on the IPSP, the depolarizing effect of intracellularly injected $Cl^-$ on $E_{IPSP}$ is considerably enhanced and the recovery time course of $E_{IPSP}$ after $Cl^-$-injections about twofold prolonged. The changes in the kinetics of $Cl^-$ extrusion across the motoneuronal membrane (as determined by the recovery of the IPSP) closely parallel the changes which $NH_4^+$ produces on $E_{IPSP}$. The findings are corroborated by observations made on the inhibitory responses of crayfish stretch receptor cells[2]. $E_{IPSP}$–$E_M$, which is strictly negative at normal $E_M$ ($Cl^-$-free electrodes), is reduced by about $^3/_4$ when 5 m*M* (2,5%) $Na^+$ is replaced by $NH_4^+$. The limit of the depolarizing movement of $E_{IPSP}$ was again found to be $E_M$. With and without externally added $NH_4^+$, $E_{IPSP}$ and $E_M$ follow different time courses if external $[K^+]$ is altered. These results further indicate that $E_{IPSP}$ depends of an electrochemical gradient which is not directly related to the $K^+$ gradient. In both preparations, the results with external $NH_4^+$ suggest a block of an active chloride extrusion mechanism which normally provides the ionic gradient for hyperpolarizing synaptic inhibition.

[1] H. D. Lux, C. Loracher and E. Neher, Expl Brain Res. *11*, 431 (1970).
[2] H. Meyer and H. D. Lux, in preparation.

### Further Evidence for an Existence of Separate and Independent Sodium and Potassium Channels in the End-Plate Membrane

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To investigate whether the transmitter induced conductance change in the subsynaptic membrane consists of separate and independent sodium and potassium channels ($\Delta G_{Na}$ and $\Delta G_K$), the effects on the EPP of lidocaine derivative, 2-Diethylamino-N-cyclohexyl-acetamide (10666), were studied and compared with those of procaine. 10666 ($10^{-4}$ w/v) depressed the amplitude of EPPs, and accelerated the initial rate of decay of the EPPs. The development of the secondary long-lasting plateau was not so marked as with procaine. As a result, a characteristic hump never appeared in the EPPs recorded in 10666. However, the EPCs obtained at the normal levels of the resting potential were almost indistinguishable from the corresponding EPCs recorded in $10^{-4}$ procaine. Thus, in cases of procaine and 10666, the same EPCs generated EPPs of different wave form. No significant differences were noted in the membrane constants of muscle fiber between two cases. Since drug effects on $\Delta G_{Na}$ can be represented approximately as those on the EPCs recorded at the resting potential, and in cases of procaine and 10666 the effects are identical, the present results should be explained by a different drug action on $\Delta G_K$. In fact, the time course of the EPCs recorded at the sodium equilibrium potential, which reflects that of $\Delta G_K$, was found to alter differently with the two drugs; it did not prolong in procaine whereas a long-lasting slow phase appeared in 10666.

### Release of Neurotransmitters and Amino Acids from Isolated Cerebral Tissues

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Isolated neocortical or piriform cortical tissues from guinea-pigs and rats were incubated in a superfusion system[1] in which they could be electrically stimulated. Uptake and efflux of several compounds have been examined, together with some associated changes in the tissues themselves. $^{14}$C-adenine and -adenosine mixed with part of the endogenous adenine nucleotides of the tissue and yielded $^{14}$C-adenosine triphosphate; on stimulation, adenosine was released and contributed to augmented formation of cyclic adenosine monophosphate[2–5]. Actions of noradrenaline and of inhibitory agents on these processes will be described; noradrenaline and serotonin were released concomitantly.

The tissues are capable of maintaining concentrations of free amino acids close to those of the brain in situ, measured as total α-amino nitrogen. Nevertheless they have a considerable output of amino acids[6] such that the quantity of free amino acid in tissue plus superfusing fluid increases 3- to 6-fold during 1–2 h incubation in the case of glycine, valine, leucine, isoleucine and tyrosine. The total output of amino acids was increased by electrical stimulation; after prior uptake of isotopically labelled valine and leucine, output of these compounds also was increased by stimulation.

Extracellular fluids thus receive from tissues of the brain, many compounds which are general metabolites in addition to those with specific roles in transmission. This may be related to trophic effects exerted postsynaptically, and also to the economy of neurons with elongated axons or dendrites, in which centrifugal movement of materials preponderates over centripetal: the counter-movement being by metabolites extracellularly.

1 H. McIlwain and S. H. Snyder, J. Neurochem. *17*, 521 (1970).
2 S. Kakiuchi, T. W. Rall and H. McIlwain, J. Neurochem. *16*, 485 (1969).
3 A. Sattin and T. W. Rall, Molec. Pharmac. *6*, 13 (1970).
4 H. Shimizu and J. Daly, Biochim. biophys. Acta *222*, 465 (1970).
5 I. Pull and H. McIlwain, unpublished (1971).
6 D. A. Jones and H. McIlwain, J. Neurochem. *18*, 41 (1971) and unpublished.

### The Antagonism of Glutamate Action at Central Neurones

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The actions of glutamic acid and a number of its analogues and derivatives as excitants of spinal neurones were first described some years ago, and substantially similar results have since been obtained at several other locations in the central nervous system. To date only N-methylaspartic acid has been suggested as an antagonist of glutamate action; however this compound is itself powerfully excitatory, and it appears that blockade of the effect of glutamate could be produced only when sufficient methylaspartate had previously been administered to cause a depolarization block of the neurone.

In the present experiments glutamate derivatives which themselves have little or no excitatory action have been examined as possible antagonists of the excitations produced by glutamate, homocysteate or acetylcholine at single neurones of nuclei ventralis lateralis and ventralis posterolateralis thalami of anaesthetized cats. The substances were delivered electrophoretically from multibarrelled micropipettes and records made of the extracellular action potentials of the cells. Several compounds were inactive; however two (DL-α-methylglutamate and the diethyl ester of L-glutamate) reversibly prevented the excitatory effect of glutamate applied from another barrel of the electrode assembly at more than half of the cells tested. At the doses used the compounds were without effect upon the background firing rate of the cells, and the responses to homocysteate and acetylcholine were usually either less attenuated or completely unaffected. The synaptic activation of neurones in nucleus ventralis posterolateralis elicited by stimulation of peripheral nerves was also antagonized by these substances.

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### The Release of $^{14}$C-Glycine from Electrically Stimulated Rat Spinal Cord Slices

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There is now much evidence that glycine and γ-aminobutyric acid (GABA) are inhibitory transmitters in the central nervous system[1,2] but it has proved difficult

to demonstrate changes in the release of these amino acids from the brain after nerve stimulation. This difficulty may be due to the efficient uptake mechanisms for glycine and GABA which are present in the spinal cord and brain[3–5]. In recent studies, electrical stimulation of cortical slices and the cerebral cortex in vivo has been shown to increase the release of GABA from the cortex[6,7], but the release of amino acids from the spinal cord has not yet been demonstrated. In the present experiments, the release of $^{14}$C-glycine, $^{3}$H-GABA, $^{14}$C-glutamate and $^{3}$H-alanine from superfused slices of spinal cord has been studied using a method similar to that described by SRINIVASAN et al.[6].

After the first 10 min of superfusion there was a steady spontaneous efflux of $^{14}$C-glycine from the slices of spinal cord. Stimulation of the tissue by rectangular pulses (60 Hz, 20 mA, 5 m/sec) for 30 sec caused as ignificant increase in the efflux of radioactivity, the maximum increase being 2.3 times the resting efflux (mean of 8 experiments). Similar results were obtained with $^{3}$H-GABA and $^{14}$C-glutamate, the maximum evoked releases being about 2 and 3 times the prestimulation value respectively. However, the increased efflux of glycine was not a nonspecific effect on the cell membrane, as electrical stimulation did not cause an increased release of $^{3}$H-alanine or $^{14}$C-urea from cord slices.

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1 R. WERMAN and M. H. APRISON, in: *Structure and Functions of Inhibitory Neuronal Mechanisms* (Pergamon Press, Oxford and New York 1968).
2 K. KRNJEVIĆ, Nature, Lond. *228*, 119 (1970).
3 M. J. NEAL and H. G. PICKLES, Nature, Lond. *223*, 679 (1969).
4 L. L. IVERSEN and M. J. NEAL, J. Neurochem. *15*, 1141 (1968).
5 M. J. NEAL, J. Physiol., Lond., in press (1971).
6 V. SRINIVASAN, M. J. NEAL and J. F. MITCHELL, J. Neurochem. *16*, 1235 (1969).
7 L. L. IVERSEN, J. F. MITCHELL, M. J. NEAL and V. SRINIVASAN, Br. J. Pharmac. *38*, 452 (1970).

## Ultrastructure of Transmitter Release

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The presynaptic membrane complex consisting of triagonally arranged dense projections and the hexagonal 'presynaptic vesicular grid' raises the question of additional substructures facilitating transmitter release. In freeze-etched neuropil of the mammalian central nervous system, one finds membrane faces belonging to vesicle-containing nerve terminals which have resisted fracturing and continue to adhere to underlying nerve cell processes. There are reasons to believe that these membrane areas are presynaptic. They are characterized by a high density of membrane particles and show a varying number of circular protrusions (as seen from the inside of the nerve terminal) which appear as small pits from the outside of the terminal bag. The protrusions may bear an opening on top so that they are comparable to endo/exocytotic stomata in nonsynaptic membranes. However, in contrast to the latter (which measure 350 Å in diameter) the presynaptic protuberances have an outer diameter of only about 200 Å at their base. These structures are designated 'synaptopores'. Pattern analysis reveals that they occupy a varying proportion of nodal points of a hexagonal pattern corresponding to the geometry of the 'presynaptic vesicular grid'. Synaptopores seem to represent a synapse-specific, reversible form of close contact between vesicles and presynaptic membrane which may facilitate the release of transmitter from the vesicles into the synaptic cleft. Increasing doses of pentobarbital or pretreatment with tetanus toxin reduce the number of protuberances per square unit of presynaptic membrane. Thus, the synaptopores may represent an index of synaptic activity.

## Acetylcholine and Central Synaptic Transmission

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Although it has long been hypothesized that acetylcholine (ACh) could be a central synaptic transmitter, clear evidence in support of this proposal has only recently become available. The discovery of interneurons (Renshaw cells) within the spinal cord which were excited by ACh released from presynaptic terminals of the recurrent collaterals of motor fibres was a striking confirmation both of the existence of central cholinergic mechanisms and of the prediction that an axon should release the same transmitter at all of its terminals. Studies on the distribution of ACh and its related enzymes, which support the concept that cholinergic synapses are widely distributed throughout the central nervous system, have now been extended by experiments on ACh release and on the effects of iontophoretically applied ACh and its antagonists. Release, which can be correlated with stimulation of neuronal pathways, has been demonstrated from the cerebral and cerebellar cortices, the thalamus, caudate nucleus and spinal cord. Excitant actions of ACh have been demonstrated on neurones in many areas of the brain and in certain instances, such as in the cerebellum, thalamus, geniculate and caudate nuclei, this effect has been related to the presence of cholinergic synaptic receptors. Two excitatory receptors, one with nicotinic and the other with muscarinic properties, have been reported on the Renshaw cell. Muscarinic receptors on cortical Betz cells appear to mediate their action by reducing membrane potassium conductance. Depressant actions of ACh have also been recorded in various areas of the brain and spinal cord. In the cerebral cortex and caudate nucleus this action is apparently mediated through synaptic receptors.

## Mechanisms of Storage of Catecholamines in Subcellular Organelles

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Catecholamines and nucleotides, e.g. ATP form aggregates of high apparent average molecular weight in aqueous solutions as demonstrated by analytical ultracentrifugation. The aggregation increases with rising concentration of the solutes and with decreasing temperature. Bivalent cations (Ca, Mg) in low concentrations promote this aggregation, whereas high amounts of these

metal ions as well as amine liberating drugs (tyramine, amphetamine) cause desaggregation. Gelatine, the physicochemical properties of which are similar to those of the chromogranins, seems to associate with the norepinephrine-ATP-Ca aggregates, whereas the globular protein albumine does not interact with these aggregates.

The water-soluble constituents of isolated bovine adrenal medullary granules contain aggregates of high apparent average molecular weights the size of which increases with decreasing temperature. According to sedimentation velocity experiments followed by individual analysis of the low molecular weight compounds, these aggregates consist mainly of adrenaline, noradrenaline and ATP.

It is concluded that in adrenal chromaffin granules the catecholamines are stored in the form of high molecular weight aggregates with nucleotides and that the chromogranins may serve as a matrix to which these aggregates are reversibly bound.

## Inhibitory Synaptic Activities of Thalamic Neurons

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Inhibitory synaptic pathways in the thalamus exhibit marked heterogeneity as inferred from the characteristics of intracellularly recorded IPSPs elicited by different modes of stimulation of different thalamic neuronal subsystems. Prolonged synchronizing-IPSPs observed during spontaneous and induced electrocortical synchronization are entirely attributable to a postsynaptic conductance-increase mechanism as indicated by continuous monitoring of postsynaptic membrane resistance. Similar IPSPs are elicited in medial nonspecific neurons during low-frequency VA-VL stimulation. Thus reciprocally related internuclear synaptic pathways link specific and nonspecific thalamic nuclei albeit asymmetry is evident in respect to IPSP latency. Comparison of the mode of engagement of thalamic VL relay cells and interneurons by brachium conjunctivum (BC), ansa lenticularis (AL) and medial thalamus stimulation (MTh) indicates significant convergence of AL projections to inhibitory synaptic pathways involved in MTh-induced evoked synchronizations. AL and BC afferents exhibit monosynaptic excitatory convergence onto VL-relay neurons but only BC-stimulation elicits succeeding IPSPs in these elements. Such IPSPs are not likely to result from the operation of recurrent pathways in view of this dissociation of the effects of convergent monosynaptic pathways to VL cells.

Thalamocortical radiation stimulation evokes variable latency IPSPs in VL relay cells exhibiting antidromic spikes. Only early (40–60 msec) phases of such IPSPs produce variable blockade of antidromic spikes. During later phases of IPSPs such spikes develop prolonged depolarizing after-potentials some of which are associated with inactivation responses.

Several varieties of IPSPs are observed in lateral geniculate neurons. In addition to the usual smoothly summating prolonged IPSPs seen in other thalamic neurons in rare instances LGB neurons may exhibit spontaneous brief IPSPs which interrupt the rising phase of small, rapid depolarizing potentials. These are not elicited by photic stimulation which generally initiates prolonged IPSPs and suppresses the spontaneous brief 'biphasic' PSPs. The latter may represent the activity of reciprocally related dendrodendritic synapses, although other mechanisms are not excluded.

## Evidence for the Existence of Different Types of Receptor for Glycine

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Strychnine blocks motoneurone inhibition induced either recurrently by Renshaw cells or by glycine, indicating that glycine mediates recurrent inhibition (CURTIS 1969). Renshaw cells also mutually inhibit each other by a postsynaptic mechanism (RYALL 1970) and Dale's hypothesis predicts that the transmitter is also glycine. Glycine inhibited Renshaw cells and was readily antagonized by strychnine. However, the mutual inhibition was surprisingly resistant to strychnine administered either microelectrophoretically or intravenously. Occasional nonspecific effects were produced but only when the strychnine ejecting current was much greater than that required to abolish the inhibition by glycine. Bicuculline or picrotoxin intravenously or bicuculline microelectrophoretically did not specifically antagonize the mutual inhibition.

One explanation of strychnine-resistant mutual inhibition could be that Renshaw cells liberate different transmitters at different terminals. Since there is no positive evidence in favour of this hypothesis, it is rejected. There is evidence against the possibilities that a) the inhibitory synapses are located remotely from the soma of the Renshaw cells and b) strychnine is acting presynaptically at some terminals only. A last possibility, which is analogous to the demonstrated presence of two types of acetylcholine receptor on Renshaw cells, is that there are also both strychnine-resistant and strychnine-sensitive glycine receptors on Renshaw cells. Strychnine-resistant receptors may also be present on other central neurones where they may account for some strychnine-resistant postsynaptic inhibitions.

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## Excitatory Synaptic Transmission in the Cat's Superior Cervical Ganglion in situ

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The transmission of preganglionic reflex discharges through the superior cervical ganglion (SCG) was studied in cats anaesthetized with chloralose. The preganglionic reflexes were induced by afferent volleys in cutaneous and muscle nerves. The characteristics of the postganglionic reflexes resembled in every respect those of the preganglionic sympathetic nerve trunk[1,2]. For instance, both kinds of discharges displayed the separation

in four components; both were elicited by activity in the same types of myelinated afferent fibres; both showed considerable variations in amplitude and latency and were augmented by tetanic stimuli. It is concluded that the preganglionic reflex components are transmitted through the SCG with a proportional input/output relationship. A similar relationship has been found for the transmission of preganglionic volleys set up by direct stimulation of the preganglionic cervical sympathetic trunk[3–5]. A comparison of these results with the present experiments reveals a striking similarity between the discharges set up by somatic nerve stimulation and those elicited by direct stimulation of the CST. From this evidence and the results of single fibre studies[6] it is postulated that stimulation of peripheral nerves evoked activity in all fibre types present in the preganglionic trunk, and that this activity was transmitted through the SCG in much the same fashion as the preganglionic volleys induced by direct electrical stimulation of the CST. Thus it appears that little modification of somatically induced sympathetic reflex discharges takes place at the sympathetic ganglia which, under these conditions appear to act solely as relay stations in the efferent pathway.


1 R. F. Schmidt and K. Schönfuss, Pflügers Arch. ges. Physiol. *314*, 175 (1970).
2 A. Sato and R. F. Schmidt, Pflügers Arch. ges. Physiol., in press (1971).
3 G. H. Bishop and P. Heinbecker, Am. J. Physiol. *100*, 519 (1932).
4 J. C. Eccles, J. Physiol., Lond. *85*, 179 (1935).
5 A. Rosenblueth and F. A. Simeone, Am. J. Physiol. *122*, 688 (1938).
6 W. Jänig and R. F. Schmidt, Pflügers Arch. ges. Physiol. *314*, 199 (1970).


## Mechanisms of Monoamine-Induced Depression of Cortical Neurones


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A variety of amines, including noradrenaline (NA), 5-HT and histamine, depress the activity of cortical neurones when applied to them by iontophoresis. This depression might occur indirectly through the release of inhibitory amino acids. Thus the amines might excite inhibitory interneurones, causing GABA release onto the neurones under study, as proposed for the noradrenaline-induced depressions in the olfactory bulb[1]. However, in the cerebral cortex, monoamine-induced depression is relatively resistant to conventional NA and 5-HT antagonists, although these are effective in blocking monoamine-induced excitation[2]. Amines might possibly trigger the release of inhibitory amino acids within brain tissue in some less specific fashion. However, 5-HT-induced depression of cortical neurones was not obviously affected by applications of strychnine which clearly prevented the depressant effects of glycine on the same cells[3] and experiments are in progress with bicuculline and picrotoxin, which are much more effective antagonists of the 'GABA like' amino acids. The effect of amines on the release of inhibitory amino acids from brain slices is being studied. Preliminary experiments show that NA $10^{-4}M$ has no clear effect on the resting efflux of $^3$H-GABA from cortical slices in vitro and studies on the effects of monoamines on the evoked release of GABA are in process.


1 D. Felix and H. McLennan, Brain Res. *25*, 661 (1971).
2 E. S. Johnson, M. H. T. Roberts, A. Sobieszek and D. W. Straughan, Int. J. Neuropharmac. *8*, 549 (1969).
3 E. S. Johnson, M. H. T. Roberts and D. W. Straughan, Br. J. Pharmac. *38*, 659 (1970).


## Heterosynaptic Facilitation


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Heterosynaptic facilitation was first described by Kandel and Tauc[1] in *Aplysia* neurones. It was defined as an increase in amplitude of a test excitatory postsynaptic potential (EPSP) after the activation of a pathway (heterosynaptic pathway) different from that which produced the test EPSP. Later on Epstein and Tauc[2] were able to produce heterosynaptic facilitation under conditions which excluded the participation of posttetanic potentiation.

Direct evidence has now been obtained that heterosynaptic facilitation and posttetanic potentiation represent two distinct phenomena. There are two identified neurones, both situated in the left pleural ganglion; one, an interneurone, produces an EPSP in the other, the so-called left giant cell. Recording simultaneously from both, we observed that heterosynaptic facilitation of the EPSP occurred in the absence of spike activity in the test interneurone.

The heterosynaptically induced increase of the EPSP is specific to given types of input; other types of postsynaptic potentials are not affected[3].

Iontophoretic injections of 5-hydroxytryptamine at critical sites in the neuropile of the ganglion, presumably representing the location of the nerve terminals of the test interneurone on the giant cell produced an increase of the test EPSP similar to that induced by heterosynaptic facilitation. The possible participation of 5-hydroxytryptamine as a natural transmitter of heterosynaptic facilitation will be considered.


1 E. Kandel and L. Tauc, J. Physiol., Lond. *181*, 28 (1965).
2 R. Epstein and L. Tauc, J. Physiol., Lond. *209*, 1 (1970)
3 T. Shimahara and L. Tauc, J. Physiol., Paris *62*, 449 (1970).


## Neurally-Mediated Induction of Specific Enzymes in Peripheral Adrenergic Neurons; a Model for 'Trophic Effects' of Neuronal Activity


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It is well-known that the response of one neuron to the activity of another is not only confined to rapid, transitory effects such as changes in ionic-permeability of the neuronal membrane or changes in glucose- or $O_2$-consumption, but that it can also involve long-lasting alterations in structural elements of the neuron. These so-called 'trophic effects' of neuronal activity are generally both inadequately defined and poorly understood.

Recent experiments have shown that the peripheral adrenergic neurons (including the chromaffin cells of the adrenal medulla) lend themselves favourably for the study of such 'trophic effects'. A prolonged increase in the activity of the pre-ganglionic sympathetic fibres (produced by cold exposure or drugs) induces an increased synthesis of tyrosine hydroxylase and dopamine-$\beta$-hydroxylase, whereas other enzymes involved in the biosynthesis (dopa-decarboxylase) or metabolic degradation (monoaminoxidase) of catecholamines remain unchanged. Similar changes occur in specific regions of the CNS after prolonged increase in activity. The possible implications of this selective, trans-synaptic induction of enzymes is discussed in connection with problems of ontogenesis, retention of information in the CNS and the development of drug-tolerance not related to induction of drug-metabolizing enzymes.

## Mechanisms of Synaptic Excitation in Sympathetic Ganglion Cells

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Synaptic excitation results from an increased ion conductance of postsynaptic membrane at virtually all chemically transmitting synapses that have been investigated. During such typical EPSPs, membrane resistance is decreased. The equilibrium potential for the increased ion conductances involved is in the depolarizing direction so that electrical hyperpolarization of the membrane increases the EPSP, whereas progressive depolarization decreases and then reverses the EPSP to a hyperpolarizing potential. Like other EPSPs, the fast EPSP in frog sympathetic ganglion cells manifests these properties. The postsynaptic ion conductance is increased by the action of the transmitter, acetylcholine, on nicotinic membrane receptors.

In addition to the fast EPSP, repetitive preganglionic B fiber stimulation generates in B ganglion cells a muscarinic slow EPSP with unique properties. In contrast to the increased conductance associated with other known EPSPs, membrane conductance (measured by a constant current pulse) decreased significantly during the slow EPSP. Furthermore, contrary to the effect of electrical membrane polarization on other EPSPs, depolarizing current increased the size of the slow EPSP, while hyperpolarizing current decreased the slow EPSP. Further hyperpolarization reversed the slow EPSP to a hyperpolarizing potential. The reversal potential of the slow EPSP was close to the $K^+$ equilibrium potential. This data strongly suggested the slow EPSP was generated by a decrease in $K^+$ conductance, but did not exclude the possibility of a decrease in $Cl^-$ conductance. However, removal of extracellular $Cl^-$ from the Ringer bath had no significant effect on the slow EPSP, excluding the possibility that an inactivation of $Cl^-$ conductance plays a significant role in the generation of the slow EPSP. On the basis of these data, we propose that the slow EPSP is generated by an inactivation of resting $K^+$ conductance.

## The Stoichiometry of Transmitter-Receptor Interactions

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Analysis of the relation of conductance change as a function of drug concentration indicates that the behavior of steady-state conductance in low concentrations provides evidence as to the number of molecules of drug that interact with a receptor in order to produce that change. Analysis of effects of higher concentrations can then provide details about the specific physico-chemical laws of interactions. The use of repetitive current ramps in an ohmic range of membrane properties also provides kinetic data.

The analysis was successfully applied to a GABA inhibitory synapse in locust muscle where data showed that three molecules of transmitter interact with the receptor and are blocked by one molecule of an antagonist, picrotoxin. The system can be described by the equation:

$$\gamma = (G/K_1)^3 \,/\, [1 + (G/K_1)^3 + P/K_2] \,.$$

Kinetic data allow determination of diffusional limitations on the system, and temperature dependence of the system. Dissociation constants, 50% reaction concentrations, free energy and heats of activation and entropy have been calculated. The values obtained are remarkably consistent and appear quite reasonable.

## Changes of Cutaneous Receptive Fields and Interaction of Dorsal Horn Neurones Induced by Microelectrophoretic Application of Amino Acids

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By means of microelectrophoretically applied excitatory (glutamic acid/homocysteic acid) and inhibitory (glycine/GABA) amino acids the extent of excitability changes of dorsal horn cells in the spinal cord was studied in cats. The size of their cutaneous receptive fields and sensitivity to mechanical stimulation were tested. All grades of excitability changes could be observed, e.g. cells previously unexcitable even by heavy mechanical stimuli (noxious) responded to hair-movement and light touch under small doses of glutamic acid (or homocysteic acid). Under glycine (or GABA) the opposite effect was observed: cells responding to lightest mechanical stimulation of the skin could be activated only by heavy pressure onto previously highly sensitive center and their cutaneous receptive field shrank concomitantly. This method proved to be a valuable tool for studying subliminal figures extracellularly. The majority of cells giving origin to the spino-cervical-tract showed a particular reaction to glutamic acid, whereas the reaction upon glycine resp. GABA was regular. These cells started firing on a quite normal glutamate dose level but reduced firing at higher doses, while their synaptic excitability decreased. From simultaneous recordings obtained with twin multibarrelled electrodes it is very suggestive that inhibitory interneurones, activated by the spread of glutamic acid are responsible for this effect. Intracellular recording showed that this particular type of cell could be depolarized by glutamic acid. Data about possible differences between these cells and other neurones of this region will be discussed.