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Recent advances in the use of selective neuron-destroying agents for neurobiological research

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During the last few years, the use of selective neuron-destroying agents has become one of the most widely used approaches in neurobiological research. The general aim of investigators using neuron-destroying agents is to provoke a more or less extended, but anatomically verifiable, neuronal degeneration and to study the consequences of the lesion from anatomical and/or functional points of view. Neuron-destroying agents are powerful tools to investigate many basic aspects of brain organization but, at the same time, they give rise to several problems related to the mechanism of action of the drugs used and to possible drawbacks in the interpretation of the results.

The aim of the present paper is to present some of the more important findings obtained, in recent years, from the experimental use of neuron-destroying agents and to make, whenever possible, a critical appraisal of the potential usefulness and drawbacks for each compound. Our attention will be primarily focussed on agents producing degeneration of nerve cell bodies; we will not make any attempt to review the extensive literature concerning the action of drugs having a primary neurotoxic action towards chemically-characterized nerve endings, such as 6-hydroxydopamine,6-hydroxydopa,5,6-and 5,7-dihydroxytryptamine[2,57,65,66,80,99,112,131,132,155].

Kainic acid

Since the discovery of the neuroexcitatory[60,144] and neurotoxic[23,54,110] action of kainic acid on invertebrate and vertebrate neurons, this drug of algal origin has been extensively used in experimental neurobiology. In the present paper we will not consider in detail the bulk of earlier findings, adequately reviewed in previous publications[20,87]; we will rather focus our attention on those aspects which are more relevant to the problem of the

relation between the mechanism of action of kainic acid and its use as a selective neuron-destroying agent.

Kainic acid is a conformationally restricted analogue of glutamic acid[20,87], which is thought to be a naturally occurring excitatory neurotransmitter[30]. The primary effect of kainic acid on sensitive neurons is a strong excitation that can be electrophysiologically monitored by measuring membrane depolarization and recording spike discharge[14,60,126]. In some systems the excitatory potency of kainic acid was found to be a hundred times higher than that of the natural neuroexcitants, glutamate and aspartate[142]. While with low doses the effect was reversible, higher concentrations of kainic acid caused an immeasurable increase of membrane conductance and the neurons became irreversibly depolarized[14,74]. It was initially proposed that the binding of kainic acid to the post-synaptic glutamate receptor could trigger the overexcitation and, eventually, the death of sensitive neurons[54,110]. Further studies, however, suggested the existence of specific binding sites for kainic acid in membrane preparations from mammalian and avian brain[24,52,77,89,97,145,146] as well as in a synaptosome-enriched particulate fraction from the goldfish brain[92]. More recently, the existence of pre-synaptic, in addition to the post-synaptic, receptors for kainic acid has been postulated[34,44,154].

In some systems, the neuroexcitatory and neurotoxic effects of kainic acid appeared to depend on the integrity of excitatory afferents (some of which were presumably glutamatergic) or on the administration of exogenous glutamate[5,7,8,14,52,68,95,150]. In other systems, however, such a relationship could not be demonstrated[6,72,119,126,142] and both the neuroexcitatory and neurotoxic effects of kainic acid occurred independently of the functional integrity of afferent systems or exogenous glutamate. Furthermore, in an in vitro preparation of the goldfish optic tectum, glutamate exhibited a moderately competitive action against the neuroexcitatory action of kainic acid, monitored by the increase of CO_2 production from tectal slices incubated in the presence of exogenous glucose[91].

These discrepant results may be explained[19,34] by postulating that the post-synaptic receptors are responsible for the direct excitatory and neurotoxic effects and that pre-synaptic receptors cause the release of endogenous excitatory transmitters, which enhance the excitatory and neurotoxic action of kainic acid. Still, one has to be aware that the situation at the receptor level may be even more complicated. In fact, in studies on mammalian brain it was possible to demonstrate specific binding sites for kainic acid in membrane preparations subjected to different kinds of manipulations, but not in intact synaptosome preparations[97]. The negative results with mammalian synaptosomes and brain slices have recently been confirmed in our laboratory. In contrast, intact synaptosomes or brain slices from the goldfish brain and spinal cord are able specifically to bind kainic acid, with the level of binding being 1 or 2 orders of magnitude higher than that found in membrane preparations from mammalian brain[92]. These preliminary observations raise exciting possibilities for future work aiming at the explanation of several discrepant results obtained with kainic acid in relation to differences in animal species used, neuronal systems investigated and experimental approach adopted.

In the mechanism recently proposed by Coyle[19] to explain the action of kainic acid, the activation of post-synaptic receptors as well as the release of endogenous excitatory neurotransmitters caused by the activation of pre-synaptic receptors would increase the level of free intracellular Ca^{++}. The high Ca^{++} level in the post-synaptic neuron would activate Ca^{++}-dependent proteases while the neuronal depolarization would increase energy consumption and decrease energy stores[7,8,19]. This model seems to explain adequately several results obtained with the in vivo administration of kainic acid[5,8,52,68,95,122,150] as well as some in vitro results[7,14,34,44]. However, this hypothesis cannot readily explain the differences in binding characteristics and in metabolic response to kainic acid observed in fish and mammalian brain preparations[7,91,92,97,118,154]. Moreover, the physiological evidence of pre-synaptic receptors for kainic acid should be tested in synaptosome preparation rather than in intact slices[34,120].

On the basis of the above results and of studies including pharmacological manipulation[31,39,51,149,165], the neurotoxic action of kainic acid on a given neuron can be regarded as the net result of the overexcitation caused by kainic acid, the reinforcement by excitatory afferents and the possible modulation by intrinsic and/or extrinsic inhibitory circuits.

While the mechanism of action of kainic acid still requires better clarification, its value as a selective neuron-destroying agent is well documented by hundreds of papers published during the last decade. The neurotoxic effect, demonstrated in a number of brain regions in organisms from fish up to mammals, results in the degeneration of sensitive neurons[17,21,23,37,42,50,53,54,70,90,107,123,124,136,150,157,158] in times ranging from some hours to few days. In some systems kainic acid destroys all the neurons present in the area of the injection site[16,21,23,163] while, in other regions, the drug is selectively neurotoxic towards specific neuron types only. Neuronal populations differentially sensitive to kainic acid have been demonstrated, for instance, in the cerebellum, the hippocampus, the habenula and the nucleus of the diagonal band of Broca[16,18,36,53,54,129].

Examples of the extremely selective neurotoxicity of kainic acid have been observed among the cerebellar Purkinje cells and the periventricular neurons of the optic tectum in the goldfish[157,158]. From an experimental point of view, it is interesting to learn that, in several systems, the neurotoxic action of kainic acid appeared to be dependent not only on the dosage used but also on the exact method of anesthesia adopted[3,19,31,39,58,114,164]. The use of different types and doses of anesthetic and tranquilizers offers, therefore, an auxiliary way to obtain selective neurotoxic effects in various nervous regions. While in mammals the neurotoxic effect of kainic acid leads to irreversible neuronal loss and to compensatory glial proliferation[20,21,23,54,72,86], there is some evidence that a partial recovery from the neuronal damage can occur in the fish brain[17].

It was originally suggested that kainic acid spared afferent terminals and axons of passage in the injected region[21,136]. Subsequent reports cast some doubt on the

axon-sparing character of kainic acid lesions[71,84]. However, several further pieces of research have given convincing evidence that kainic acid neurotoxicity does not actually affect afferent systems and axons of passage[10,17,53,104,157,158]. The selectivity of the lesions caused by kainic acid makes it possible, in many cases, to overcome the problems inherent in conventional lesion methods and makes it possible to study features of the intrinsic organization of discrete brain areas as well as parameters related to connections between anatomically-linked nuclei.

A major problem concerning the use of kainic acid lesions in experimental neurobiology derives from observations suggesting that, in some systems, the neurotoxic effect leads to neuronal degeneration at the injection site as well as in distant areas[4,21,95,129,141]. These remote lesions have been demonstrated after kainic acid injections in the hippocampus and amygdaloid complex[4,95,129,130] and have been attributed to epileptiform activity and seizures induced by the drug. In a more comprehensive report[141] based on kainic acid injections in several brain areas, it was suggested that the remote lesions reflected anatomical connections between the injected and the distant area. In other systems, however, distant neuronal damage was not reported[27,53,157,158,163] or was excluded by specific histological controls[18]. Moreover, distant neuronal damage after intracerebral injections of kainic acid appears to be dependent on several factors such as the animal species used, the drug dosage, the route of administration and the protocol of anesthesia[47,71,94,130,164,165].

On the basis of the available data it seems safe to conclude that investigators should be aware of the possible occurrence of distant lesions after localized injections of kainic acid and that histological controls should be performed when starting a lesion study with kainic acid. In particular, those brain areas which, on the basis of the known anatomical data are most likely to lead to false interpretation of the results, must be carefully checked.

Ibotenic acid

In an attempt to overcome the problems related to epileptogenic effects and to distant lesions caused by kainic acid, other potential neurotoxins have been tested. Ibotenic acid, an acid amino acid structurally similar to kainic acid extracted from toxic fungi of the genus *Amanita,* has proved to be an effective neuron-destroying agent when injected intracerebrally[43,69,134]. The neurotoxic potency of ibotenic acid appears to be lower by at least 1 order of magnitude than that of kainic acid, and considerably higher doses must therefore be used in order to obtain lesions comparable in size[43,45,46,134]. A major advantage in the use of ibotenic acid is the apparent absence of epileptogenic activity and of distant lesions outside the locus of direct administration[45,46,67].

On the basis of such observations, ibotenic acid has been recommended as a substitute for kainic acid as a selective neuron-destroying agent[46]. However, relatively few studies based on the use of ibotenic acid have been performed as yet, and there is therefore little information concerning some essential parameters of the mechanism of action of this neurotoxin. For instance, the physiological action of ibotenic acid on sensitive neurons is far from having a satisfactory explanation. In fact, some studies indicate that ibotenic acid may preferentially bind to the aspartate receptor[73]. However, in a study on the electrophysiological action of ibotenic acid on the crayfish neuromuscular junction[143] it was suggested that ibotenic acid could act through the postsynaptic (and maybe also the pre-synaptic) GABA receptors. The same discrepancy was apparent as far as the physiological action of ibotenic acid was concerned. In the crayfish neuromuscular junction ibotenic acid had no appreciable depolarizing effect[143] while, in other systems, it showed strong excitatory[73], or mixed excitatory and inhibitory, actions[98].

A recent report[67] has compared the neurotoxic effect of kainic acid and ibotenic acid in several brain regions. From the results obtained, it appears that some differences in the mechanism of action of the 2 neurotoxins must exist. It is not clear, however, whether these differences concern basic or accessory aspects of the neurotoxic action. In evaluating the usefulness of ibotenic acid as a selective neuron-destroying agent, a crucial point is that we have no information on the possible side-effects of injecting the large amount of ibotenic acid required to obtain a sizable neurotoxic effect (usually 5–20 µg as compared with 0.5–1 µg of kainic acid). Ibotenic acid, in fact, cannot be metabolized and it is likely that it does accumulate in brain tissue for rather long periods, as demonstrated for kainic acid[31].

Quinolinic acid

Quinolinic acid (2,3 pyridine dicarboxylic acid) is an endogenous compound belonging to a family of tryptophan metabolites, the kynurenines. This conformationally restricted analogue of glutamate and aspartate is a powerful excitant of central neurons when delivered by iontophoresis[114,115]. Intrastriatal injections of high doses of quinolinic acid (up to 600 nmoles) caused large destruction of striatal neurons, fall of intrinsic neurotransmitter-related markers and apparent sparing of axons of passage and afferent terminals[139,140]. No distant lesions were observed outside the injected brain areas. Intrahippocampal injections in excess of 60 nmoles led to degeneration of all the neurons present in the injected area while lower doses exhibited a preferential toxicity towards pyramidal neurons (in particular those of the CA1 zone)[139,140]. Generalized convulsions only occurred after intrahippocampal injections of at least 500 nmoles of quinolinic acid. Similar, but less pronounced, neurotoxicity was exhibited by the quinolinic acid analogue cis-2,3-piperidine dicarboxylic acid[38].

In a further histological study[137] differential vulnerability to quinolinic acid was demonstrated in various brain areas. While basal ganglia and hippocampus were very susceptible to the neurotoxic action, the cerebellum, the substantia nigra, the amygdala, the medial septum and the hypothalamus appeared more resistant. In in vitro cultures, quinolinic acid was neurotoxic towards striatal explants when cultured together with frontal cortex, but not towards striatal explants alone[159]. Similar in vitro results had been previously demonstrated for kainic acid. In preliminary studies performed

in our laboratory (unpublished observations) quinolinic acid proved to be highly neurotoxic when injected in the rat frontal cortex. Doses of 60–90 nmoles caused unselective brain tissue destruction around the injection site and high mortality within a few days after the operation. However, lower doses of quinolinic acid (15–30 nmoles) were apparently ineffective in causing detectable brain lesions.

From the few data so far available, quinolinic acid appears to be a promising neurotoxin for neurobiological research. It shares some of the neurotoxic properties of kainic acid[139, 140] but, like ibotenic acid, does not seem to cause generalized epileptiform activity and distant damage. The neurotoxic action of quinolinic acid requires, of course, to be tested in other nervous regions and in other animal species. From our preliminary results on the cerebral cortex it appears that there should be a remarkable degree of differential sensitivity to quinolinic acid neurotoxicity among different nervous regions. The selective neurotoxic effect would be the result of differences of the neuroexcitatory action of quinolinic acid towards various neuronal populations[114, 115]. The physiological and experimental conditions which give rise to this differential sensitivity require careful investigation. A major problem with the use of quinolinic acid is the high dosage required to obtain a significant neurotoxic effect. In contrast with ibotenic acid, quinolinic acid is metabolized and therefore its actual concentration in the brain tissue is probably decreased significantly during the injection and subsequent diffusion. Studies on the mechanism of action, on the rate of metabolization and the metabolic fate of the injected quinolinic acid are essential in order to make a better assessment of its usefulness as a selective neuron-destroying agent in neurobiological research.

Glutamate

A neurotoxic effect of systemically injected monosodium glutamate in specific brain areas was originally demonstrated by Olney[102] in infant mice, and has subsequently been confirmed in other species[12, 111, 116]. The neurotoxic effect was restricted to some hypothalamic areas, in particular the arcuate nucleus, and to a few other brain regions. Glutamate neurotoxicity has also been demonstrated in primary spinal cord cultures derived from fetal mice[11].

In adult animals, intracerebral injections of very high doses of glutamate only caused very limited damage around the injection site[86, 108, 138]. The relative inefficacy of glutamate as a neurotoxic agent is probably due, at least in part, to the fact that a very active uptake system exists in brain tissue and, therefore, the effective concentration of glutamate is readily reduced at the injection site. Consistent with this explanation is the fact that previous transection of presumedly glutamatergic afferents significantly increases glutamate neurotoxicity[138], most likely as a consequence of the reduced glutamate uptake.

Pyroglutamic acid

While previous studies[20, 25] did not reveal any significant excitatory action or binding antagonism of L-pyroglutamic acid, an intermediate amino acid of the gamma-glutamyl cycle, a recent report[125] led to different and quite interesting conclusions. Intrastriatal injections of pyroglutamic acid in the mouse caused postural disturbance and increased firing of sensitive neurons followed by sudden cessation of neuronal discharge. Subsequent morphological examination showed selective degeneration of striatal neurons while afferent fibers and terminals were spared. These results have encouraged new efforts to test pyroglutamic acid in other brain regions.

Folates

Folic acid and some of its metabolic derivatives have convulsant properties[9, 26, 56]. Since folates are released by nerve endings under stimulation[13] and, in cerebellar membranes, methyltetrahydrofolate is an effective competitor for the kainic acid binding site[128], it has been proposed that these compound are endogenous neuroexcitatory and possibly neurotoxic agents. In agreement with this hypothesis, folate levels in the cerebrospinal fluid are more than doubled after epileptic seizures[13]. However, experiments carried out on slice preparations of the prepyriform cortex[1, 127], failed to confirm the supposed excitatory properties of folates. Binding experiments showed differential patterns of competition with kainic acid in membrane preparations from different brain areas of mammals[78] and in a synaptosome-enriched fraction from the goldfish brain[92].

In a recent paper[33] based on a wide range of physiological and biochemical tests, it was concluded that some folates may have a weak neurotoxic effect, but that this effect is not mediated through specific receptors for kainic acid. This notion is consistent with binding experiments on synaptosome preparation from the goldfish brain, recently performed in our laboratory[92]. Folic and dihydro folic acid showed a low degree of competition towards the kainic acid binding site while methyltetrahydro folic and folinic acid were ineffective.

When injected in the amygdala, striatum and substantia innominata, folic acid (50–200 nmoles) caused sustained epileptiform activity accompanied by a disseminated pattern of distant brain damage while little or no damage was apparent at the injection site[88, 108, 109]. Formyltetrahydrofolate showed similar effects while methyltetrahydrofolate had a markedly lower neurotoxic action[108]. The distant damage caused by folates was primarily apparent in brain areas anatomically connected with the sites of injection and was thought to be the result of the seizures caused by the drug administration[108, 109].

Our limited knowledge of the neurotoxic properties of folates does not allow a definitive assessment of their value as selective neuron-destroying agents. The possibility of destroying neurons in brain areas anatomically connected with the injected region raises interesting opportunities for studies focussed on neuronal connectivity. On the other hand, the complicated pattern of distant damage caused by folates and the degree of reproducibility of the neurotoxic effect obtained should be rigorously tested before one accepts the use of folates as selective neuron-destroying agents.

Dipiperidinoethane and cholinergic agents

Systemic administration of dipiperidinoethane, a piperidine derivative, caused seizures and brain damage similar to those caused by systemic or intramygdaloid injections of kainic acid[106]. It was later ascertained that the actual neurotoxic action was caused by an oxidized derivative, since intramygdaloid injections of N,N-dioxydipiperidinoethane reproduced the syndrome caused by systemic injections of dipiperidinoethane[103]. Unlike kainic acid but like the folates, the oxydized dipiperidinoethane caused relevant distant brain damage associated with limited brain damage at the site of injection. Since the molecular structure of the drug resembles that of the cholinergic agent oxotremorine, several acetylcholine agonists and cholinesterase inhibitors were subsequently tested[105]. Charbacol and neostigmine were the most effective agents, while acetylcholine itself caused seizures and brain damage only when administered together with an ineffective dose of neostigmine.

A possible explanation of these results is that the excitation of amygdala neurons containing acetylcholine receptors triggers locally seizures leading to neuronal damage in distant areas anatomically connected with the injected region. As for the case of folates, the use of the drugs described in the present chapter as selective neuron-destroying agents is made difficult by the fact that they do not cause restricted lesions at the injection site and that the distant lesions probably cannot be controlled to the extent required for neuroanatomical or neurochemical studies. On the other hand, these studies may have a clinical relevance since they give interesting information on the neuronal mechanisms underlying human diseases like epilepsy.

Colchicine

It has recently been shown that colchicine is neurotoxic when injected in the striatum[93] and hippocampus[40]. In the hippocampus a preferential neurotoxicity against the granule cells of the fascia dentata was noticed[40]. Subsequently, selective colchicine neurotoxicity was demonstrated in several other brain regions[41]. Recently, in our laboratory, we have attempted to use colchicine neurotoxicity in order to confirm some projections in the septo-habenulo-interpeduncular system[156]. By means of stereotaxic injections of µg amounts of colchicine and subsequent electron microscopic examination of the injected and the target areas, we were able to identify the medial habenula as the source of the most common types of interpeduncular terminals, the S and crest synapses. In addition, a previously controversial projection from the triangular nucleus of the septum to the interpeduncular nucleus[15,83,153] was confirmed. The cholinergic nature of the projection from the supracommissural septum to the interpeduncular nucleus and the habenula, previously suggested on the basis of a lesion study[16], has been recently confirmed using stereotaxic colchicine injections (Fonnum and Contestabile, in preparation). However, in these studies it was observed that, as would be anticipated on the basis of the available reports in the literature[28,49,162], colchicine caused damage or degeneration of axons crossing the injected areas.

It was concluded that colchicine cannot be used as a neuron-destroying agent in the absence of controls allowing exclusion of erroneous interpretations of the results caused by degeneration of fibers of passage. This is, of course, a serious limitation of the use of colchicine for neuroanatomical and neurochemical purposes. It remains, however, interesting for neurobiologists to try to explain why colchicine displays such a remarkable degree of selectivity against some specific neuronal types[40,41,156]. From some recent studies it appears that colchicine neurotoxicity is a consequence of the binding of colchicine to tubulin and that the selective neurotoxicity may be due to a more stable bond in some neuronal elements, possibly in connection with increase of neuronal firing and induction of epileptiform activity[41,79,152]. An additional neurotoxic effect of colchicine may be due to some pre-synaptic action on Ca^{++} influx and transmitter release, as suggested by a recent in vitro study[101].

Lectins

Neurotoxic properties of some lectins, proteins of plant origin such as ricin, abrin and modeccin have been recently demonstrated[148,151,160]. Intraventricular injections of a very small amount of ricin (0.04 µg/rat) resulted in a significant inhibition of protein synthesis by brain tissue, and a dose as low as 0.084 µg/rat caused the death of 50% of the experimental animals within 7 days after surgery[151]. The application of ricin, abrin and modeccin to the trunk of the peripheral nerves resulted in the degeneration of parent cell bodies[160]. The cause of the neuronal degeneration can be attributed to the inactivation of polyribosomes in the cell bodies by the retrogradely transported toxin[29,32,135,160]. Previous experiments, in fact, demonstrated that ricin, injected in the anterior eye chamber or in the submandibulary gland, was taken up by terminals of the postganglionic sympathetic neurons and retrogradely transported to the parent cell bodies.

The possible occurrence of «suicide transport» of lectins[160] in central nervous pathways would raise very interesting opportunities for experimental neurobiology. By injecting ricin or other toxic lectins in a given brain area, it would be possible to cause neuronal degeneration in brain regions projecting to the injected area. However, a recent paper[161] failed to show retrograde transport of ricin conjugated to horseradish peroxidase from the injection site to neuronal cell bodies in the projection area. A possible explanation of the different ability to transport lectins in peripheral and central systems may reside in the different binding of the molecule to surface glycoproteins in peripheral and central nerve terminals[161]. In our laboratory, we have tested the possibility of obtaining neuronal degeneration after retrograde transport of ricin injected in the rat striatum (unpublished observations).

From a set of preliminary experiments, it appeared that an extensive degeneration area was readily observed at the injection site using ricin doses as low as 10 ng. The effect on areas projecting to the striatum such as the frontal cortex and the substantia nigra showed, however, a large variability. No neuronal degeneration was

usually observed in the substantia nigra while neuronal loss in the frontal cortex was well evident in a few cases but not appreciable in others. These results raise several unanswered questions on the possibility of using toxic lectins as neuron-destroying agents and further researches in this field are clearly needed.

Other toxins

Bipiperidil mustard, a bipiperidine derivative, systemically administered to rats, has been shown to provoke neuronal lesions restricted to some areas of the ventromedial hypothalamus, the dorsal vagal nuclei and areas of the medial and supracommissural septum[75,76]. No information is available on the mechanism of action of this substance.

Ethylcholine mustard aziridinium ion intraventricularly injected in mice caused a remarkable decrease of high affinity choline uptake in the cerebral cortex and hippocampus; no such effect was apparent for the striatum[81]. It was proposed that a toxic effect of the drug against the cholinergic terminals could be the result of the interference with the high affinity uptake system present in the same terminals[35,81]. In situ injections have been subsequently reported significantly and selectively to reduce cholinergic parameters in the striatum[22]. In a subsequent work performed on the hippocampus[82], ethylcholine mustard selectively reduced high affinity choline uptake and choline acetyltransferase while pre-synaptic noradrenergic and serotoninergic markers appeared unaffected. Since AChE activity, which is considered to be largely linked to extrinsic cholinergic terminals in the hippocampus[100,166], did not decrease under the same experimental conditions, the authors concluded that degeneration of cholinergic synapses did not actually occur. Rather, inactivation of choline carrier at the uptake sites and some form of interaction with choline acetyltransferase, were suggested in order to explain the decrease of cholinergic markers. Finally, a cytotoxic effect against a neuroblastoma x glioma hybrid cell line was recently reported[133].

Since these cells have both an active choline transport system and high levels of choline acetyltransferase, the effect appeared again to be specific for cells provided with the most common markers of cholinergic activity. The use of ethylcholine mustard seems to be promising for the experimental study of cholinergic systems. A preliminary step to be performed, however, is a careful examination of the actual damage to cholinergic neurons and the degree of selectivity of the toxin.

Drugs acting during development

Drugs interferring with mitosis, such as methylazoxymethanol acetate and cytosine arabinoside, are able to kill dividing brain cells selectively[63]. When administered during a given foetal or post-natal period, these drugs destroy the neuroblasts undergoing division in the hours (about 12 h for methylazoxymethanol acetate) following the treatment with the result that these neuronal populations are eliminated from the adult brain. Methylazoxymethanol acetate and cytosine arabinoside have been successfully used to ablate neuronal populations from the cerebellum and cerebral cortex[61,64,85,147]. By such an experimental approach the degranulated mouse cerebellum was studied with respect to the modification of neurochemical parameters associated with the underdevelopment of granule cells[62]. Methylazoxymethanol acetate administration at the 15th day of gestation in pregnant rats resulted in selective elimination of neuronal populations from cortical layers II, III and IV, and in the modification of the balance between GABAergic and noradrenergic parameters in the adult cortex[62,63].

The use of the drugs described above seems a very useful tool for the study in adult animals of the effect of the exclusion of large neuronal populations during development. These effects can be better studied in such regions as the cerebral and cerebellar cortices in which the process of neuronal maturation is delayed in comparison with the brainstem and the basal nerve centers. This is a very favorable condition since most of the brain nuclei projecting to the cerebral and cerebellar cortices have completed their cellular divisions and are not affected by the drug administration. The situation would probably be more complicated for brainstem and basal nuclei since the generation time of neurons in these areas largely overlaps. The possibility that the drug administration on a given gestational day affects neurons in many, and possibly interconnected, nuclei, would considerably increase the difficulty of interpretation and make it rather hard to obtain clear-cut and informative results.

It has recently been observed[59,96,113] that neonatal administration of capsaicin, a drug extracted from the red pepper, results in selective degeneration of peptidergic terminals reaching the dorsal horn of the spinal cord through the dorsal root. Substance P and somatostatin are among the neurotransmitters and/or neuromodulators involved in the function of the affected neurons[96]. The mechanism of action of capsaicin has not yet been clarified. Recent observations[113] indicate that the neurotoxic action may be the result of the interference of capsaicin with the nerve growth factor, known to contribute to the maturation of substance-P containing primary sensory neurons.

Beta-bungarotoxin, isolated from snake venom, exhibited peculiar neurotoxicity towards some types of neurons and synaptic terminals in the chick embryo. In particular, a potent neurotoxic effect was demonstrated during development or in cultures, against sensory neurons of the spinal ganglia, motor spinal neurons, ganglion and amacrine cells of the retina[55,117,121]. In the chick retina the neurotoxic action appeared to be selectively restricted to cholinergic and GABAergic neurons since marker enzymes for these neurons were dramatically reduced while markers for other neuronal and glial enzymes were unaffected[121]. The only report concerning beta-bungarotoxin action on adult mammalian brain[48] indicates a selective neurotoxicity towards cholinergic and GABAergic systems in the rat hippocampus. The results obtained with beta-bungarotoxin are stimulating and seem worthy of more systematic studies in order to characterize the action of this neurotoxin better, both at developmental stages and in adult animals.

Discussion

As stated in the introductory remarks, the primary aim of the present paper is to give a concise review of recent results obtained by using neurotoxins as selective neuron-destroying agents. The neurotoxins taken into account are very different as far as their chemical nature and their supposed mechanism of action are concerned. Several of them are exogenous substances while others are substances normally present in the brain. It is, however, interesting to point out that the entire group of exogenous excitotoxins[20] (kainic acid, ibotenic acid and related substances), primarily act on sensitive neurons by mimicking, in an amplified manner, the action of endogenous substances and, in particular, of excitatory neurotransmitters. A common property of the toxins considered in the present paper is that all of them can be used to provoke in mature or in developing brain discrete and, to some extent, selective neuronal lesions. They therefore constitute a very powerful tool to investigate the anatomical organization of the brain as well as the neurochemical basis of its function. Furthermore, several neuron-destroying agents act by interfering with some step(s) of the normal neuronal metabolism and, therefore, the full explanation of the neurotoxic mechanism would improve our understanding of the physiological function of nerve cells. This aspect seems particularly relevant for drugs interacting with natural neurotransmitter systems at the level of pre-synaptic recognition sites for high affinity uptake and release control and/or at the level of post-synaptic receptors responsible for direct neuronal excitation.

It is clear from the present paper that the neurotoxins considered vary greatly in their degree of selectivity towards specific neurons. Drugs acting during development, such as methylazoxymethanol acetate, have no selectivity towards specific neuronal types but are extremely selective towards dividing neuroblasts. On the contrary, catecholamine and serotonin neurotoxins only destroy catecholaminergic and serotoninergic neurons[66]. These drugs were the first to be used as denervation tools and their degree of selectivity towards specific neuronal types seems still to be unsurpassed. From the bulk of the experimental data reviewed in the present paper, it can be predicted that the future interest of investigators will be mainly focussed on neurotransmitter specific neurotoxins or, at least, on neurotoxins able to destroy specific types of neurons selectively. Ethylcholine mustard and beta-bungarotoxin are interesting examples of neurotoxins having a possible selective action towards specific neurotransmitter systems (cholinergic and, maybe, GABAergic). The selectivity of their action, however, requires a more complete evaluation by combined anatomical and neurochemical studies.

The specificity of action of excitotoxins is not absolute with respect to the neurotransmitter systems of sensitive neurons. In addition, the neurotoxicity of these substances seems, in many cases, to be the result of a cooperative effect between the sensitivity of particular neurons and the overall excitatory input converging on the same neurons.

From the results so far available, colchicine seems to be rather selective in its neurotoxic effect towards different neuronal populations. This selectivity cannot be easily explained on the basis of the available data on the effect of the drug on axonal transport and microtubule assembly. A possible explanation of the selective neurotoxic effect of colchicine may reside in the impairment of some basic aspect of neuronal metabolism. This metabolic impairment would be more dangerous, and would eventually result in death, for those neurons less well-provided with energy stores and/or with higher levels of metabolic activity.

Capsaicin has proved to be a rather selective neurotoxic agent towards peptidergic neurons of the spinal ganglia, particularly in the immature animals. This drug, on the other hand, has no neurotoxic effect towards central peptidergic neurons in mature brain.

In many cases, the selectivity of neurotoxins appears to be linked to the presence of specific recognition sites on neuronal membranes. For some of the neurotoxins considered in the present paper, specific binding sites have been characterized while for other neurotoxins the presence of such recognition sites can be postulated in order to explain the neurotoxic effect. On the contrary, drugs such as methylazoxymethanol acetate, lectins and, possibly, colchicine probably do not require a specific recognition site on neuronal membranes in order to exert their neurotoxic action.

The use of powerful neurotoxins for experimental neurobiology usually requires careful interpretation of the results and accurate control for the actual brain lesion by anatomical techniques. This is a quite obvious suggestion but, sometimes, such anatomical controls are not performed to the necessary extent in investigations primarily concerned with the modification of neurochemical markers in the injected brain area and in its terminal projection fields. In some cases, the effectiveness of the lesion is simply inferred by the modification of neurochemical markers in the injected brain area and in its terminal projection fields. This procedure does not give information concerning the actual extension of neural damage and the possible differences in drug sensitivity among different types of neurons present in the injected area. The first point, i.e. lesion extension, is of primary interest in order to evaluate the neurochemical consequences of the neurotoxic effect in the injected area as well as in its terminal projection fields. The second point, i.e. differences in neuronal sensitivity to the injected drug, is very important too since it can give information on the modifications of neurochemical markers preferentially linked to specific neuronal populations.

The problem of the anatomical control of the lesions is particularly relevant when dealing with kainic acid and related excitotoxins. The use of these drugs, in fact, may have 2 major drawbacks. First, the extension of the lesion at the injection site is sometimes rather variable in different animals possibly due to differences in the individual sensitivity to the drug and/or to the anesthetic used. Second, kainic acid and other excitotoxins may cause neuronal damage not only at the injection site but also in distant areas as a consequence of their epileptiform activity. These remote lesions, when present, may considerably complicate the interpretation of the results. The above-mentioned factors strongly suggest

that extensive histological controls should be performed by using Nissl stained sections. Specific staining for degenerating neurons and electron microscopic examination may be useful in order to check the possible occurrence of remote lesions and the selective neurotoxic effect towards specific neuronal types. A time-consuming but valuable procedure to examine simultaneously histological damage and changes of neurochemical markers (e.g. neurotransmitter related enzymes such as choline acetyltransferase, glutamate decarboxylase and acetylcholinesterase) is to use relatively thick (about 40 µm) cryostat sections. Some of the sections are stained and used to check the actual neuronal damage while other sections are freeze-dried and used for enzyme assays (Contestabile and Fonnum, in preparation).

As stated in some of the previous paragraphs, the neuronal degeneration caused by neurotoxins is followed by a more or less evident glial reaction which can be easily monitored in Nissl stained sections by the increase of glial cells in areas previously filled by the degenerated neurons. The phase of glial proliferation occurs during the first week after the lesion and it probably constitutes a permanent alteration of the anatomical organization of the affected area. Optimal survival time after neurotoxin lesions is usually a few hours to 3 days for the study of initial structural alteration and of the degeneration process, and 5–10 days for studies focussed on the modification of neurochemical markers. Longer survival times can be used but, in this case, investigators should be aware of the possibility that the results may, at least in part, reflect the occurrence of anatomical and neurochemical rearrangement of the lesioned area as a consequence of plasticity phenomena (for instance, sprouting of nerve terminals). Evidence of partial anatomical and neurochemical recovery after kainic acid administration has been found in a long term study on the goldfish optic tectum[17]. It is likely that the capacity for partial recovery is much lower in mammals than in lower vertebrates. As a concluding remark, it seems clear from the present review that the capabilities of selective neuron-destroying agents have not yet been exhaustively applied to neurobiological research. In fact, most of the drugs listed in the preceeding paragraphs are still in an early stage of experimentation and can be viewed, at present, as promising tools for future investigations. It may be predicted that new substances having specific neurotoxic effect will be introduced in future years. The results so far obtained by using selective neuron-destroying agents strongly suggest that the use of new types of neurotoxins will substantially improve our understanding of basic neural mechanism and brain function.

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