shock seizures. Anticonvulsant activity was defined as prevention of the tonic extensor seizure and toxicity induced by any or all of the convulsant stimuli, unless otherwise noted. Four animals were tested at each of four to six different doses of each anticonvulsant drug. Dose ranges were 0.7 to 3.2 mmole/kg for ethosuximide, 1.1 to 4.4 mmole/kg for α-DMGBL, and 0.5 to 3.9 mmole/kg for α-EMGBL. The ED_{50} is that dose at which anticonvulsant activity was present in 50 percent of the animals. This value was determined by the method of Litchfield and Wilcoxon (*11*) and is given with the 95 percent confidence interval in parentheses.

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Functional Restoration of the Traumatically Injured Spinal Cord in Cats by Clonidine

Abstract. *The long-term, chronic, paralysis resulting from spinal cord injury in the cat has been reversed by the use of an α_2-adrenergic receptor agonist, clonidine. Administration of this drug resulted in "normalization" of sensory-motor and autonomic dysfunctions. Preliminary studies of the use of clonidine in humans with traumatically injured spinal cord indicate that autonomic dysreflexia can be controlled and spasticity minimized. The data suggest that biochemical and pharmacologic manipulation of receptors may ameliorate paralysis following traumatic injury to the spinal cord as well as to the brain and brainstem.*

In the mammalian spinal cord, most monoaminergic neuronal pathways are descending (*1, 2*). A traumatic injury, which causes the cessation of action potentials, eventual hemorrhagic necrosis of the central gray matter, and extensive structural degeneration of long tracts of the white matter within 24 to 36 hours after injury, disrupts the neuroendocrine negative feedback (*3*), receptor activation (*1*), and end-product feedback inhibition on the molecular level (*4*).

After the transection of the spinal cord, axoplasmic transport continues to the lesion site, resulting in the accumulation of serotonin (5-hydroxytryptamine), and norepinephrine above the lesion in the spinal cord (*1, 2, 5*) and brainstem (*5*) and a loss of the biogenic amines from the distal portion of the spinal cord (*2, 5*). In contrast to monoaminergic descending pathways, substance P (SP)–containing fibers in the spinal cord represent ascending spinal tracts; SP accumulates below the lesion level (*6*). Therefore, after chordotomy, the axons of descending and ascending neurons from the cells of origin to the site of the lesion remain biologically and functionally viable. Spasticity and episodic hypertensive crises (autonomic dysreflexia) are caused by noxious stimuli arising from afferent somatic and visceral inputs. These stimuli are conducted by the spinal cord below the level of the lesion (*3*).

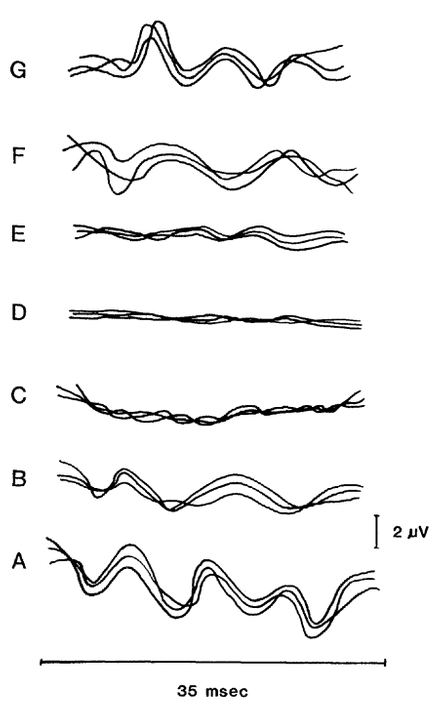

Fig. 1. In this cat the exposed dura was impacted by a 20-g weight dropped from a height of 25 cm (a 500 g-cm force). Tracings of three consecutive SEP's are shown superimposed at (A) just before, (B) 10 minutes after, (C) 20 minutes after, and (D) 30 days after impaction of the spinal cord and just before treatment. Note that SEP's are essentially absent 20 minutes after impaction and remain so for about 1 month. (E) After the first infusion of clonidine (5 μg/ml in 15 ml of saline) and administration of angiotensin II (0.1 mg/kg), SEP's were still absent. On days 2 and 3 of treatment, the animal received a 0.1-mg tablet of clonidine twice daily. On day 4 of treatment, the cat received a 0.1-mg clonidine tablet in the morning and then an infusion of clonidine and angiotensin II as on day 1. (F) Although SEP's had returned on day 4 during and after infusion, the consecutive determinations did not become constant until (G) 2 hours later. On day 5 of treatment the cat moved its tail and felt pinching by withdrawal of the hind legs. After the return of SEP's, the animals received 0.05-mg clonidine tablets four times daily. Within 4 to 8 weeks this and other cats paralyzed and treated in the same way started to walk; all tactile, thermal, pressure, and pain sensations, as well as motor coordinations, seem to have returned.

After a traumatic injury to the spinal cord, alterations of the membrane phospholipids will affect the configuration and hence the activity of the major phospholipid-dependent enzymes (*7*), as well as transmitter-receptor coupling (*8*) and the bindings of endogenous opiate receptors (*9*).

At my laboratory we have investigated membrane pathology in the traumatically injured spinal cords of cats, using physiological, biochemical (*3, 5*), immunocytochemical (*6*), and light microscopic, scanning, and transmission electron microscopic (*7*) techniques. We have also examined the pathology from the standpoint of lipid-free radical damage to neuronal membranes (*7*). It was reasoned that if functional restoration were to occur in the central nervous system (CNS), it was essential to reestablish the fluid-dynamic state of the cell membranes. As already mentioned, a significant part of the architecture of descending (*1, 2, 5*) and ascending (*6*) pathways remains intact. We postulated that a specific agonist might provide the appropriate signal or stimulus to its own membrane receptors on the descending or ascending tracts and serve as a guide for nerve tracts to grow along their genetically regulated paths.

The adrenergic system is a major descending pathway. The bulbospinal projections of the noradrenergic system, and those of the serotonergic and dopaminergic systems, project to the same or different regions of the spinal cord (*2, 10*). Therefore, the α_2-adrenergic agonist, clonidine, was chosen for our studies because it might also affect serotonin- and dopamine-containing neurons through the intimate interconnections of their circuitry in the spinal cord (*10*) and because clonidine has a high degree of selectivity for the α_2-adrenergic receptors in the CNS (*11*).

We used Allen's model of spinal cord trauma. This model is widely accepted as being the closest representative of the traumatic injury incurred by humans (*12*). Intramuscular ketamine or intravenous pentobarbital (30 mg/kg) was used to anesthetize 47 mongrel cats. Arterial blood pressure was continuously monitored in animals for at least 2 hours. After recording the stable initial arterial

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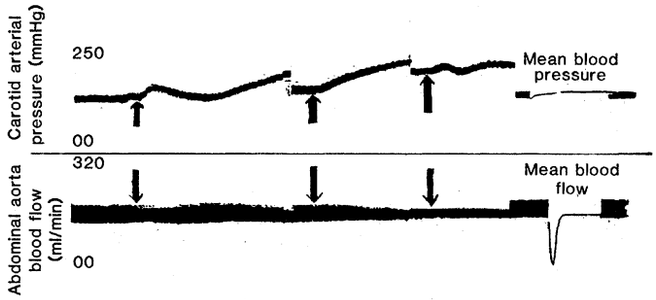

Fig. 2. Segments of a record of blood pressure and blood flow from a cat paralyzed by a 500 g-cm force applied to the spinal cord. Clonidine (5 μg/ml in 15 ml of saline) was infused very slowly 23 hours after impaction (at the first arrow). There was an immediate transitory rise in blood pressure, followed by a fall and a quick rise as the infusion continued. Thirty minutes after the infusion, the blood pressure had dropped almost to the initial level (just before the second arrow). During this interval, the clonidine had depressed the blood flow slightly. After increasing the rate of infusion (the second arrow), blood flow decreased sharply and within 5 minutes it was one-half of the initial blood flow, whereas the blood pressure had not reached the basal level. Increasing the rate of infusion of clonidine produced the third increase in blood pressure (the third arrow). Blood pressure continued dropping while the blood flow amplitude increased, with both variables reaching initial values 50 minutes after the start of infusion, when the mean blood pressure and blood flow were 118 mmHg and 138 ml per minute, respectively (see text for peripheral and central effects of clonidine).

blood pressure, a dorsal laminectomy was performed in the thoracic region of the cats from T_3 to T_5. After restoration of blood pressure, all cats were then traumatized by means of a 20-g weight being dropped from a height of 25 cm (500 g-cm force) on the exposed dura at the fourth thoracic segment. Thirty cats were not treated; the remaining 17 cats were treated at different time intervals after spinal cord contusion with clonidine (5 μg/ml in 15 ml of saline); a total of 0.075 mg of clonidine was intravenously infused. After infusion of the clonidine, angiotensin II (0.1 mg/kg) was slowly infused to bring the arterial blood pressure to the initial stabilized level. These 17 cats were also given, orally, a 0.1-mg tablet of clonidine twice the next day and were again infused with the same amount of clonidine and angiotensin II until the somatosensory-evoked potentials (SEP's) showed signs of return (Fig. 1).

To ascertain the adequacy of the trauma, the SEP's were recorded before and after impact by stimulating the sciatic nerve by means of needle electrodes inserted through the posterior thighs (*13*). The recording of SEP's from the lower extremities requires the presence of intact ascending pathways. The absence of SEP's indicates a complete disruption of the spinal cord tracts. Therefore, SEP's were also used to assess the conduction return. In all 17 cats treated with clonidine, SEP's returned; six of these cats, treated from days 14 to 48 after paralysis, walked with complete return of sensory and motor functions, including those of bladder and bowel (Fig. 1). In the 30 control cats, which were tested for 1 to 4 months after traumatization, SEP's never returned as the animals progressed from the acute, flaccid phase to the chronic, spastic, and autonomically dysreflexic phase of paralysis.

The depressive effects of clonidine were monitored by measuring arterial blood pressure and abdominal aortic blood flow (*14*). Naloxone, thyrotropic-releasing hormone (TRH), and ε-aminocaproic acid (ε-ACA) act in acute traumatic models of spinal cord injury to ameliorate the damage by causing an increase in the arterial blood flow and regional perfusion pressure (*7, 15*). In contrast, intravenous administration of clonidine has a paradoxic effect: it first increases the arterial blood pressure by acting as an α-noradrenergic agonist on the peripheral vascular receptors, and then, by virtue of its high degree of lipophylicity, it rapidly enters the CNS through the blood-brain and blood-spinal cord barriers. The central action of clonidine is to stimulate bulbospinal and spinal α_2-receptors. The central effect quickly overcomes its peripheral effect and depresses the blood pressure. If the rate of infusion is increased by a few drops, however, the response of the peripheral α-receptors to the agonist, vasoconstriction, becomes dominant over the central effects and arterial blood pressure rises, only to be depressed again as the central mechanisms take over (Fig. 2). Yohimbine and tolazoline, α_2-blocking agents, and the octapeptide, angiotensin II, antagonize the depressive effects of clonidine. The prevention of restorative capacity of clonidine by the α_2-blockers and the possible antagonism or synergism with this drug by angiotensin II is not known. This effect of clonidine in lowering the arterial blood pressure in paralyzed cats, and in quadriplegic subjects with spinal cord lesions above sympathetic outflow, indicates that in addition to the vasomotor center in the brainstem, the spinal cord sympathetic centers may also play a major role in antihypertensive and sympathoinhibitory effects of clonidine.

Histological and ultrastructural examination of the lesion site demonstrates the presence of myelinated fibers, astrocytes, oligodendrocytes, and undifferentiated cells. The origin of these fibers has not been determined.

The present work demonstrates the extreme plasticity of the CNS and introduces a new concept and approach to amelioration and restoration of function

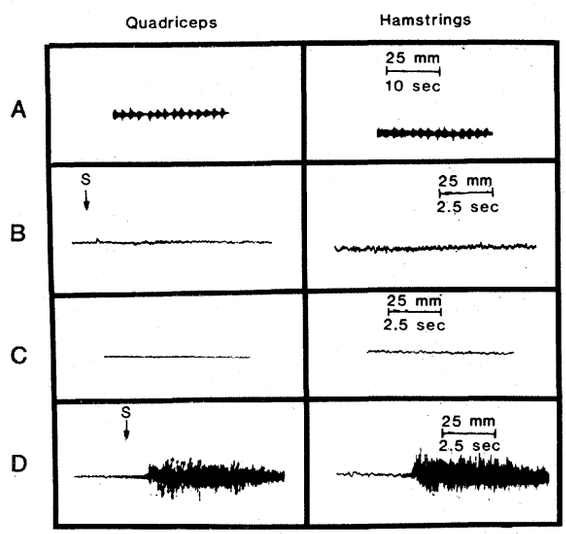

Fig. 3. The effects of clonidine on spasticity in a patient who had received traumatic injury with a C_5 "incomplete" transverse myelopathy 3½ years previously. Electromyograph (EMG) activity was recorded in the quadriceps and hamstrings regions of the same leg. Recordings were obtained (A) before and (B, C, and D) 9 days after oral administration of 0.05 mg of clonidine four times daily. When the drug reached its peak concentration in the blood, 2 hours after drug administration (B), the evoked spastic activity was inhibited. Further, when the plasma and, therefore, the central concentration of the α_2-adrenergic agonist was low, 8 hours after clonidine administration (C), spontaneous EMG activity was markedly diminished. However, the stimulus, a voluntary waving of the arms which evoked little EMG activity in (B), elicited uninhibited spastic activity in both the quadriceps and hamstrings 8 hours after treatment (D).

in the traumatically injured spinal cord of mammals, including humans. Whereas such agents as methylprednisolone sodium succinate, naloxone, TRH, and ε-ACA must be used promptly to prevent damage during the early, acute phase of injury, clonidine administration can wait until the vital signs have stabilized after spinal shock. Nevertheless, the sooner clonidine is administered the more likely it is to minimize the muscular atrophy and occurrence of other dysfunctions associated with the flaccidity of the acute phase of traumatic injury. Clonidine stimulates the α_2-receptors in the intermedio-lateral cell columns of the spinal cord and in the ventral horn which in turn inhibit γ- and α-motor neurons. The random visceral and somatic afferent inputs into the distal stump of the traumatically injured spinal cord of humans and other animals that cause autonomic dysreflexia (*3*) and spasticity (Fig. 3) are thus blocked by clonidine's tonic stimulation of α_2-adrenergic receptors.

Clonidine has been administered orally to more than 30 humans with spasticity resulting from a traumatic cervical or thoracic transverse myelopathy (*16*). The drug has served to check the potentially dangerous increases in arterial blood pressure during episodes of autonomic dysreflexia (*7*). It has also prevented the spasticity (Fig. 3) of musculature which debilitates most of these patients and frequently retards their rehabilitation. Because of the depressive effect of clonidine on sympathetic preganglionic outflow, this drug must be used with caution.

This new approach to the restoration of function in the traumatically injured CNS of mammals may find use in the immediate or delayed treatment of traumatic injuries to the spinal cord as well as brainstem lesions and cerebrovascular accidents.

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13. To record SEP's we placed the cathodes proximal to the anodes. Rectangular pulse stimuli were delivered from a Grass S2 stimulator by way of a stimulus isolation unit. A frequency of 1 Hz, with 1-msec duration, was adjusted to sufficient intensity to produce visible contractions of the lower extremity. Once set, the intensity was not changed for the rest of the experiment. Recordings were made with two vanadium screws inserted through drill holes made into the skull. One was placed in the midline over the sensory cortex for the lower extremities and the second at about 1 cm anterior to the first. A ground electrode, lightly coated with electrolyte gel, was taped over a shaved ear. Potentials were led into a Grass P511B preamplifier and magnified 20,000 times with a bandwidth of 15 to 3.2 Hz. Amplified signals were further processed through a Nicolett Signal Averager. We used a dwell time of 250 msec per point, and 65 to 100 consecutive responses were summed.
14. Carotid arterial blood pressure was measured with a pressure transducer (Statham, P23Db) attached to a four-channel recorder (Dynograph, Beckman Instruments, Inc.). Abdominal aorta blood flow was measured by means of an electromagnetic blood flow transducer and an electromagnetic blood flow meter (Biotronex Laboratory, model BL610), the output of which was connected to the same four-channel recorder.
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Polyamine Depletion Influences Drug-Induced Chromosomal Damage

Abstract. *Polyamines have been implicated in the intracellular stabilization of DNA. Depletion of intracellular polyamines influences the cytotoxicity of 1,3-bis(2-chloroethyl)-1-nitrosourea and* cis*-diamminedichloroplatinum II. By means of the sister chromatid exchange assay, it was found that intracellular polyamine depletion can also alter the induction of chromosomal damage by these cytotoxic agents.*

The polyamines putrescine, spermidine, and spermine have been implicated in the regulation of both normal and neoplastic cell proliferation (*1*, *2*). There are several proposed roles for these polycations in cellular metabolism including the stabilization of nuclear DNA (*3*). Polyamines stabilize cell-free DNA to enzymatic degradation (*4*), denaturation by x-rays (*5*), and thermal denaturation (*6*). X-ray diffraction studies suggest that primary and secondary amine groups of spermidine and spermine bind ionically to adjacent phosphate groups on one strand of DNA, and the four-carbon chain stretches across the minor groove of the double helix to form a cross bridge between phosphate groups on opposite strands (*7*). However, on the basis of the theory of counterion condensation, Bloomfield and Wilson postulate that the polyamine-mediated stabilization of DNA is the result of relatively nonspecific electrostatic interactions between polyanionic DNA and the cationic polyamines (*3*). Although the specific interactions of the polyamines and DNA have not been clearly defined, it does appear that they are important in stabilizing DNA structure. Viscoelastometry experiments indicate that there is an alteration in the conformation of DNA or its susceptibility to shear in x-irradiated cells made deficient in polyamines (*8*). Analyses of the structure of Z-DNA (*9*, *10*), indicate that spermine is located not only adjacent to the phosphate groups but also adjacent to DNA bases.

The stabilizing effect of polyamines on the structure of DNA, the probable target of many antineoplastic drugs, suggests a possible role for the depletion of polyamines in cancer chemotherapy. Cellular polyamine concentrations can

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