addition to serum proteins, we used gelatin as the outermost protein layer with little noticeable effect on cell growth.

Fluorocarbon emulsion could be stabilized with a single protein layer of, for example, serum proteins. Although some cell growth could be observed, this system did not, in general, produce a confluent cell layer on the microcarrier droplets under our experimental conditions.

Not all fluorocarbon fluids can be used for microcarrier systems by the methods we have presented. Emulsions of FC-77 could not be stabilized at the desired droplet size with polylysine and, following agitation, rapidly separated into two continuous phases. We have reported that cell spreading on planar polylysine-coated FC-77 surfaces is greatly reduced (*4*). These observations suggest that the adsorbed layer of polylysine which forms at this liquid-liquid interface has a relatively low tensile strength.

The system we have described could be significantly improved through the use of a perfusion or stirring arrangement to facilitate nutrient and waste exchange. Using other oils and adjusting the density of the beads by combining different oils might also be beneficial. In preliminary experiments we observed attachment and spreading of cells on emulsified silicone oil droplets; however, at present the fluorocarbon fluids seem superior both in supporting efficient cell growth and in producing stable emulsions with the desired droplet size.

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Proglumide: Selective Antagonism of Excitatory Effects of Cholecystokinin in Central Nervous System

Abstract. *Extracellular single-unit recording techniques were used to test the ability of proglumide to block cholecystokinin-induced excitation of rat midbrain dopaminergic neurons and dopamine-sensitive prefrontal cortex cells. Intravenous and iontophoretic proglumide administration consistently blocked cholecystokinin-induced excitations while having no effect on glutamic acid–induced increases in activity. This selective blockade of central cholecystokinin effects by proglumide suggests that this drug may be valuable for studying the possible role of cholecystokinin as a neurotransmitter or neuromodulator in the central nervous system.*

A gastrin-like peptide was first described in the mammalian brain by Vanderhaeghen *et al.* (*1*). Subsequent studies have shown that the carboxy-terminal octapeptide cholecystokinin (CCK) is probably the fragment of gastrin active in the central nervous system (*2*), and that this peptide is localized in vesicles and released by calcium-dependent mechanisms (*3*). By means of immunofluorescence histochemical methods it has been shown that CCK coexists with dopamine (DA) in a subpopulation of midbrain neurons (*4*). In addition, both systemic injection and local microiontophoretic application of CCK increase the neuronal activity of DA cells (*5*) in midbrain regions where CCK-immunoreactive DA neurons occur (*4*). Recently, the glutaramic acid derivative, proglumide, which has been used in the treatment of peptic ulcers in Europe (*6*), has been shown to be an antagonist for CCK receptors in the pancreas (*7*). Here we report that proglumide selectively blocks CCK-induced excitations of both DA neurons in the ventral tegmental area and non-DA neurons in the prefrontal cortex.

Male albino rats (Charles River, 250 to 370 g) were prepared in accordance with the guidelines of the American Physiological Society. All surgery was performed on rats under temporary halothane anesthesia. A tracheotomy was performed on each animal which was then mounted in a stereotaxic apparatus. A hole was bored in the skull over both the prefrontal cortex [anterior 9650 to

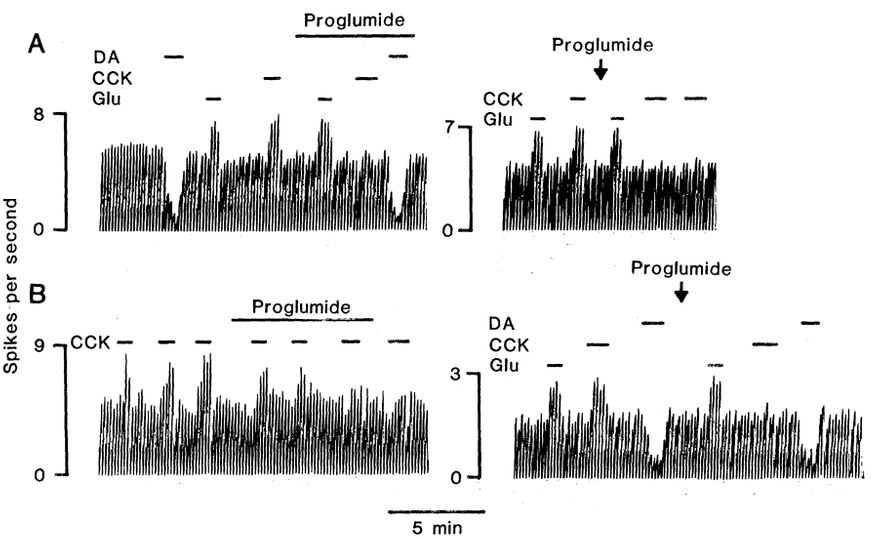

Fig. 1. Typical blocking effects of proglumide on CCK-induced changes in the activity of dopamine innervated prefrontal cortex neurons and midbrain dopaminergic neurons. (A) Cumulative rate histograms of the spontaneous activity of DA-sensitive prefrontal cortex neurons. (Left) Iontophoresis of CCK (10-nA ejection current) and glutamic acid (*Glu*, 2 nA) increased neuronal activity whereas DA (25 nA) inhibited cell activity. Local application of proglumide (30 nA) selectively antagonized the CCK-induced increases in firing rate. (Right) Iontophoretic application of CCK (15 nA) and glutamic acid (3 nA) increased cell activity. Only the CCK effect was blocked by the intravenous administration of proglumide (arrow, 0.2 mg/kg). (B) Cumulative rate histograms of the spontaneous activity of midbrain DA neurons. (Left) Iontophoretic application of CCK (30 nA) reproducibly increased neuronal discharge and the concurrent ejection of proglumide (50 nA) blocked this effect. (Right) Local application of CCK (10 nA) and glutamic acid (3 nA) excited neuronal activity whereas DA (20 nA) inhibited firing. Intravenously administered proglumide (arrow, 0.2 mg/kg) selectively blocked the excitatory effect of CCK. Horizontal bars represent the onset and duration of iontophoretic drug application.

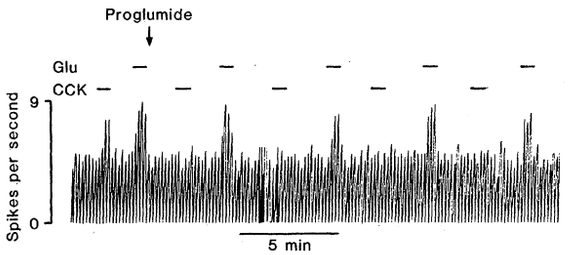

Fig. 2. Cumulative rate histogram illustrating the typical long-lasting antagonism of CCK-induced changes in neuronal activity. Iontophoretic application of CCK (15 nA) and glutamic acid (*Glu*, 5 nA) increased the activity of this spontaneously active midbrain DA neuron. Intravenously administered proglumide (arrow, 0.2 mg/kg) selectively antagonized the effects of CCK for the duration of the recording. Horizontal bars represent the onset and duration of iontophoretic drug application.

11,050 μm; lateral 400 to 1200 μm (*8*)] and the ventral tegmental area (anterior 1610 to 2420 μm; lateral 0 to 1000 μm). All wound edges and blunt pressure points were infiltrated with a long-acting local anesthetic (mepivacaine hydrochloride). The animals were then paralyzed with gallamine triethiodide (20 mg/kg), respired with room air, and allowed to recover from the initial halothane anesthesia for at least 30 minutes before the start of each experiment. Body temperature was monitored and maintained at 36° to 37°C.

Single-cell electrical activity was recorded through the center barrel of a five-barrel micropipette that was filled with 2*M* NaCl containing 2 percent Pontamine sky blue (impedance in vitro at 135 Hz, 4 to 8 megohms; overall tip diameter, 4 to 7 μm). Three of the four outer barrels contained one of the following drugs: the sulfated, COOH-terminal octapeptide cholecystokinin (CCK-S, 10 μg/ml, *p*H 8.0; Squibb); dopamine hydrochloride (DA, 0.1*M*, *p*H 4.0; Sigma); monosodium glutamate (0.1*M*, *p*H 8.0; Sigma); and proglumide (DL-4-benzamido-*N*,*N*-dipropylglutaramic acid, 10 μg/ml, *p*H 8.0; Robbins). The fourth barrel was filled with 3*M* NaCl and used for the automatic neutralization of tip currents (*9*). Retaining currents of +10 nA (−10 nA for DA) were applied to all drug barrels to eliminate the passive efflux of drug. Extracellular electrical signals were led into a high-input impedance amplifier (bandpass settings: 100 Hz to 3 kHz) whose output was fed to a storage oscilloscope and audio monitor. Spontaneous firing rates were calculated on-line by a rate-averaging computer. Dopamine-containing neurons of the ventral tegmental area and DA-sensitive prefrontal cortex cells were identified by previously described electrophysiological criteria (*10*). Recording sites were marked by passing a −30 μA current through the center barrel for 10 minutes. The animals were then perfused with a 10 percent buffered Formalin solution, their brains were removed, and frozen sections were cut (50 μm). The sections were stained with a formal-thionin solution and examined with a light microscope for the location of the ejected dye.

All DA neurons sampled in the ventral tegmental region (N = 28) were excited by the iontophoretic application of CCK-S (ejected at −3 to −40 nA) as well as glutamate (−1 to −10 nA). In contrast to the immediate excitation produced by glutamate, the CCK-S–induced increases in activity showed a delayed onset (10 to 30 seconds). Iontophoresis of proglumide (−5 to −30 nA), while having no effect on basal discharge rates, totally blocked the excitatory effects of CCK-S on all neurons tested (N = 16), but had no effect on glutamate-induced increases (N = 7; Fig. 1). Similar selective antagonism of CCK-S effects was observed with the intravenous administration of proglumide (N = 5; 0.1 to 0.2 mg/kg; Fig. 1). All neurons sampled in the prefrontal cortex (N = 13) were also excited by the local application of both CCK-S and glutamate. Again, the onset of the glutamate effects were immediate, whereas the CCK-induced increases in firing developed over 10 to 30 seconds. Proglumide (administered systemically, N = 4, or iontophoretically, N = 9) again was able to block the CCK-S–induced increases in neuronal activity while having no effect on either glutamate-induced excitations or basal firing rates (Fig. 1). In all instances, regardless of the route of administration, the antagonistic effects of proglumide lasted, without decrement, for at least 20 minutes (Fig. 2).

The present results demonstrate that the glutaramic acid derivative proglumide can selectively antagonize the excitatory effects of iontophoretically applied CCK-S on both A10 DA neurons and DA-sensitive neurons in the prefrontal cortex. Proglumide appears to be equally effective in this respect whether administered systemically or iontophoretically. The latter finding suggests that its action is at sites located on or near the neurons being sampled. The delayed onset of action for iontophoretically applied CCK-S could be due to any one or a combination of factors including (i) a site of action some distance from the pipette tip, (ii) a weak action necessitating a high concentration to be effective, (iii) a metabolite being the active agent, and (iv) the slow ejection of this peptide from the glass micropipette. The lack of recovery of CCK's excitatory effect after proglumide administration (for example, 20 minutes after the offset of proglumide iontophoresis) is puzzling. One possible explanation for this finding would be irreversible binding of proglumide to CCK receptors.

The ability of proglumide to selectively block the excitatory effect of CCK on neurons of the central nervous system suggests that it may be useful for studying the function of CCK in the brain and its possible role in normal and abnormal behavior. For example, since it appears that CCK is involved in satiety (*11*), it is possible that proglumide exerts an opposite action, that is, induces eating. If this were true, proglumide might be a potentially useful therapeutic agent in the treatment of anorexia. Similarly, since CCK coexists within and excites midbrain DA neurons (*4*, *5*), and a hyperfunctioning of central DA systems is hypothesized to be involved in schizophrenia, it would be of interest to ascertain the actions of proglumide in individuals afflicted with this disorder.

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Midbrain Microinfusions of Prolactin Increase the Estrogen-Dependent Behavior, Lordosis

Abstract. *Microinfusions of rat prolactin into the dorsal midbrain of estrogen-treated, ovariectomized rats increased lordosis behavior. Midbrain microinfusions of antiserum to prolactin into rats displaying maximum lordosis had the opposite effect. The distribution of a prolactin-like substance in the brain was studied immunocytochemically. The results suggest that a hypothalamic neuronal system projecting to the midbrain contains a prolactin-like substance that plays a role in facilitating this behavior and therefore may mediate some of the effects of estrogen on the brain. These data, together with others from studies of the prolactin gene and its regulation, indicate that it may be possible to analyze a sequence of molecular events in the brain that facilitate a behavioral response.*

Since estrogen alters brain functions, one can postulate the existence of specialized nerve cells that respond to changes in circulating estrogen concentrations and transduce this information into a form recognizable by other nerve cells. A useful model of an estrogen-modulated brain function is lordosis, a behavior displayed by sexually receptive rats. Much of the neural circuitry controlling lordosis has been determined (*1*). Cells in the mediobasal hypothalamus facilitate lordosis (*2*) and partially mediate the effect of estrogen on this behavior (*3*). Some of the estrogenic effect appears to be mediated by the synthesis of messenger RNA and protein (*4*). Projections from cells in the mediobasal hypothalamus to the dorsal midbrain are critical for lordosis (*5*). Some of these hypothalamic cells have midbrain projections and respond directly to estrogen (*6*), but the chemical nature of the neuroactive substances produced and released by these cells is unknown. Some cells within this anatomical area contain a prolactin-like immunoreactive substance (*7*). We show here that hypothalamic cells releasing a prolactin-like substance in the midbrain may facilitate lordosis.

Rats were ovariectomized and fitted with intracranial guide cannulas as described (*8*), but with two changes being made in the procedure: (i) the rats did not receive implanted Silastic capsules containing estradiol and (ii) the stereotaxic coordinates were 1.3 mm rostral to lambda and 4.5 mm below the dura; the cannulas, with tips 2.5 mm apart, were lowered with the top angled rostrally 10° from verticle. Infusions into control sites were made with these same coordinates, except the cannulas were lowered 1.5 mm below the dura. The microinfusions were 1 μl in volume, given over 2 minutes simultaneously on both sides of the brain.

The day after cannulation, each rat received 10 μg of estradiol benzoate (Sigma) subcutaneously. A week later, the rats were given an estrogenic regimen designed to produce low levels of lordotic responsiveness (*9*). Two days later, each rat received 1 mg of progesterone subcutaneously to ensure that each rat was capable of displaying moderate to strong lordoses. The rats were tested for lordotic responsiveness (*10*) in two tests before being injected with progesterone and at 30-minute intervals after injection, for 4 hours. Rats displaying progesterone-induced increased lordosis reflex scores (at least 1.0 point on the four-point scale) were given this same estrogenic regimen about a week later. Instead of injecting progesterone, we microinfused peptides through the guide cannulas into the dorsal midbrain (*8*). Each rat was tested for lordotic responsiveness on three tests prior to infusion and at frequent intervals afterward.

Table 1. Effect of microinfusion of 400 ng of prolactin into the cerebral cortex of ovariectomized, estrogen-treated rats ($N = 6$) and into the central gray of adrenalectomized, ovariectomized, estrogen-treated rats ($N = 3$). Values are means (± standard error) of the lordosis reflex score (*10*).

Time	Infusion into	
	Cerebral cortex	Central gray
Before infusion*	1.3 ± 0.18	1.0 ± 0
0 minute	1.4 ± 0.36	1.0 ± 0
5 minutes	1.1 ± 0.26	1.0 ± 0
20 minutes	1.3 ± 0.21	1.8 ± 0.20
1 hour	1.3 ± 0.22	2.5 ± 0.24
2 hours	1.5 ± 0.19	2.5 ± 0.29
3 hours	1.4 ± 0.19	2.3 ± 0.33
4 hours	1.4 ± 0.30	2.4 ± 0.31
5 hours	1.2 ± 0.36	1.4 ± 0.31
6 hours	0.9 ± 0.31	1.0 ± 0
9 hours	0.5 ± 0.30	0.1 ± 0.07
24 hours	0.6 ± 0.33	0.7 ± 0.33

*This test was conducted 20 to 60 minutes before the prolactin infusion, which began immediately after time zero.

Microinfusion of prolactin (NIAMDD-rPRL-B-3) significantly increased lordosis reflex scores in a dose-related fashion, with a latency of approximately 40 minutes (Fig. 1A). Maximum responsiveness was obtained 90 minutes to 4 hours after infusion. Infusion of vehicle (0.025*M* phosphate-buffered saline, *p*H 7.2) had no effect. Microinfusion of 400 ng of prolactin into the cerebral cortex overlying the midbrain ($N = 6$) had no effect on lordosis (Table 1). The behavioral effect of prolactin did not require progesterone release from the adrenal gland, since infusion of 400 ng of prolactin into the midbrain of three adrenalectomized rats increased the lordosis scores, with a timing and magnitude similar to the effect in non-adrenalectomized rats (Table 1).

To determine whether the effect of prolactin was due to contaminants of the prolactin preparation (*11*), we infused three of these contaminants into the dorsal midbrain in great excess of the amounts calculated to be present in the prolactin preparation. Neither growth hormone (NIAMDD-rGH-B-6, 25 ng/μl; $N = 4$), vasopressin (Bachem, 1 ng/μl; $N = 4$), nor oxytocin (Bachem, 1 ng/μl; $N = 3$) significantly increased the lordosis reflex scores. Infusion of adrenocorticotropin [ACTH-(1–24), Bachem, 25 ng/μl, which is approximately equimolar to 200 ng of prolactin; $N = 3$] into the dorsal midbrain had no effect on lordosis.

To investigate the physiological necessity of midbrain release of prolactin-like immunoreactive substances in the estrogenic maintenance of lordotic responsiveness, we infused undiluted antiserum to prolactin (NIAMDD-rPRL-IC-1) into the dorsal midbrain of rats displaying maximum lordoses because of prolonged estrogen administration. Infusion of the antiserum ($N = 5$) decreased lordosis reflex scores (Fig. 1B) with a latency of 10 minutes and for a duration of nearly 2 hours. Infusion of normal rabbit serum