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3. Vibrations were transduced by an accelerometer pickup (B & K Instruments type 4339) attached to the midrib (*Musa*) or middle (*Agave*) of the leaf. From the preamplifier (B & K 2625), the signal was fed to a measuring amplifier (B & K 2606) whose output was monitored with headphones and an oscilloscope (Tektronix D10) and recorded on a frequency-modulated tape recorder (SE Laboratories Eight-Four) at 19 cm/sec, along with whispered protocol on a separate channel. In other trials we recorded airborne sounds; a tripod-mounted condenser microphone (B & K 4133) and preamplifier (B & K 2619) provided the signal to the measuring amplifier. Analysis and graphing of the signals involved use of a Mini-Ubiquitous FFT computing spectrum analyzer (Nicolet Scientific Corp., model 446), an X-Y recorder (Hewlett-Packard 7045A), and a pen recorder (Gould Brush 2200). Nine male and 12 female *C. salei* from a stock of laboratory-bred spiders were used. We conducted most observations between 0800 and 1100 hours and used low levels of illumination except during filming. Behavior was filmed at 100 to 200 frames per second with a Cine-8 Super-8 camera (Visual Instrumentation, model SP-1). Microscopic examination revealed no organs specialized for sound production on any part of the spider.
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8. The mean vibration frequency was determined from the predominant peak in the power spectra. This value was corroborated by counting the number of wave peaks per unit time within each pulse on the pen recordings.
9. Since the female's pheromone is probably species-specific, there may have been less selective pressure on her to evolve a vibratory signal that is as regular as the male's.
10. The female's signal included waves at least four times greater in amplitude than those of a vigorously courting male, as recorded during the period just prior to copulation. (At that time, both spiders are on the female's leaf and roughly equidistant from the vibration pickup, the latter being on the leaf used for the male's introduction.) This amplitude difference is evident in Fig. 2B, in which an actively signaling male was recorded while still on the leaf used for introduction and only 15 cm from the pickup, while the female was on another leaf and 76 cm from the pickup (measured through the plant). Even at that distance the waves of her signal caused acceleratory amplitudes greater than those of the male's signal waves.
11. We measured the durations of the five peak-to-peak intervals separating the final six pulses of the male courtship bouts to examine this point. The last interval (0.41 ± 0.073 second, $N = 15$) was significantly longer ($t = 6.15$, $P < .001$) than the preceding intervals (0.34 ± 0.022 second, $N = 60$). That this was not due simply to a trend of increasing intervals was indicated by the lack of a significant difference ($t = 0.91$) between the first interval (0.34 ± 0.025 second, $N = 15$) and the fourth (0.35 ± 0.023 second, $N = 15$).
12. The system yielded a fairly flat response down to about 100 Hz and fell only 5 dB at 70 Hz—not rolling off until about 50 Hz (acoustically measured at the loudspeaker).
13. The high levels of airborne masking noise induced resonant vibrations in the banana plant. Such added noise in the substrate reduced the maximum distance over which the spiders' signals could be transmitted effectively. At the highest level of airborne noise (86 dB SPL), one female responded to a male courting 0.5 m away on another leaf but did not respond when the male was placed at a greater distance until the airborne noise was reduced back to 80 dB SPL. The distance limitations imposed by various levels of induced substrate noise require future study.
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Choline Stimulates Nicotinic Receptors on Adrenal Medullary Chromaffin Cells to Induce Catecholamine Secretion

Abstract. *Choline stimulated secretion of catecholamines from primary dissociated cultures of bovine adrenal medullary chromaffin cells by interacting with nicotinic receptors. Secretion was readily detected at a choline concentration of 1 millimole per liter and was maximal at 3 to 10 millimoles per liter; it was completely calcium-dependent. Further analysis suggested that choline acts as a partial nicotinic agonist.*

Reduced function of cholinergic nerves in the central nervous system (CNS) is suspected of playing a role in a variety of neurological disorders including tardive dyskinesias (*1*), Alzheimer's disease (*2*), and memory loss in old age (*3*). A potential approach to the treatment of these diseases is to increase cholinergic function. This can be accomplished by administering choline or lecithin (from which choline is derived) (*4*). Acetylcholine is synthesized from choline by the action of choline acetyltransferase in cholinergic nerves. Preliminary trials involving this strategy have met with some success (*5*), but the actual mechanisms of action are poorly understood. In addition to being a precursor for acetylcholine, choline may have direct effects on acetylcholine receptors. Recently, Krnjević and Reinhardt (*6*) reported that choline has a muscarinic effect on CNS neurons. We now report that choline interacts directly with nicotinic receptors on chromaffin cells from bovine adrenal medulla inducing catecholamine secretion.

Chromaffin cells from bovine adrenal medulla were dissociated and maintained as monolayers in 16-mm-diameter plastic wells (Costar, Cambridge, Massachusetts) for up to 21 days (*7, 8*). Cell density was 450,000 cells per well and catecholamine content was 9 to 30 nmole per well, depending on the preparation. Secretion experiments were performed at 25°C in physiological salt solution (PSS) containing 142 m*M* NaCl, 5.6 m*M* KCl, 3.6 m*M* $NaHCO_3$, 2.2 m*M* $CaCl_2$, 15 m*M* *N*-2-hydroxyethylpiperazine-*N*′-2′-ethanesulfonic acid (*p*H 7.4), 5.6 m*M* glucose, and 0.57 m*M* ascorbic acid, as described in the legend to Fig. 1.

Choline (3 m*M*) induced detectable secretion of catecholamine after 30 seconds, and secretion continued for approximately 5 minutes (Fig. 1A). The time course of choline-induced secretion was virtually identical to that of carbachol-induced secretion (*9*). Secretion was observed at 1 m*M* choline and was maximal at 3 to 10 m*M* (Fig. 1B). At higher concentrations, secretion decreased. Because 1 m*M* and 3 m*M* choline stimulated secretion in solutions in which NaCl concentrations were maintained at 142 m*M*, the secretion induced by choline was not caused by a reduction in the NaCl concentration, as has been suggested (*10*).

Secretion induced by choline was completely Ca^{2+}-dependent. The percentage of total catecholamine released into the medium after a 15-minute incubation in Ca^{2+}-free PSS containing 0.5 m*M* $MgCl_2$ was 2.2 ± 0.6 and 2.6 ± 0.2 percent in the presence and absence of 3 m*M* choline, respectively (three wells per group). In contrast, the percentage of total catecholamine in the medium after a 15-minute incubation in PSS containing 2.2 m*M* Ca^{2+} and 0.5 m*M* $MgCl_2$ was 9.7 ± 0.8 and 3.0 ± 0.2 percent in the presence and absence of 3 m*M* choline (three wells per group; $P < .01$, Student's *t*-test). Secretion induced by the physiological agonist acetylcholine or by carbachol or nicotine is also Ca^{2+}-dependent and occurs by exocytosis (*7, 9, 11*). The similar dependence of the effects of choline on Ca^{2+} suggests that choline also causes exocytosis.

Catecholamine secretion by these cells is induced by stimulation of nicotinic but not muscarinic receptors. Secretion is induced by the nicotinic agonists nicotine and 1,1-dimethyl-4-phenylpiperazinium and the mixed (nicotinic and musca-

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rinic) agonists acetylcholine and carbachol, but not by the muscarinic agonists methacholine, muscarine, or bethanechol (*7, 9, 12*). Furthermore, the nicotinic antagonist mecamylamine is at least 100 times more potent than the muscarinic antagonists scopolamine and atropine in blocking acetylcholine- or carbachol-induced secretion (*9, 12, 13*). To determine the receptor type responsible for choline-induced secretion, the effects of mecamylamine and atropine on choline-induced secretion were investigated. Mecamylamine was over 100 times more potent than atropine in blocking secretion. Thus choline stimulates secretion by interacting with nicotinic receptors.

Because the maximal amount of catecholamine secretion induced by choline (6 to 10 percent of the total catecholamine) was less than that induced by acetylcholine, carbachol, or nicotine (15 to 25 percent), it seemed possible that choline was a partial nicotinic agonist with the capacity to inhibit a full agonist such as acetylcholine. Indeed, at concentrations greater than 1 m*M*, choline inhibited secretion induced by 30 μ*M* acetylcholine (Fig. 2B). The data support the conclusion that choline interacted with the same receptor as acetylcholine and demonstrate that a concentration of choline 30 to 100 times greater than that of acetylcholine was required to directly cause secretion or to inhibit acetylcholine-induced secretion.

It is conceivable that choline was not acting directly but was transformed in the culture by choline acetyltransferase to acetylcholine. This is unlikely, however, because (i) no choline acetyltransferase activity was detected in the cultures (*14*), (ii) the time course of choline-induced secretion did not display a lag (a delay in secretion after exposure to choline would be expected if acetylcholine were being synthesized), and (iii) the presence of choline inhibited secretion induced by acetylcholine (acetylcholine synthesized from choline should not inhibit secretion stimulated by exogenous acetylcholine) (*15*).

Nicotinic stimulation in the CNS may induce a variety of effects including alteration of behavior (*16*), activation of the brainstem reticular system (*17*), and stimulation of the Renshaw cells (*18*) which, in turn, could inhibit spinal motor neurons. Nicotinic receptors are also present in sympathetic ganglia and on skeletal muscle. There are important differences in the pharmacology of nicotinic activation at the various peripheral and central sites (*19*), and the possible nicotinic actions of choline at the sites have yet to be extensively investigated.

The concentration of choline in human serum is 10 μ*M* and rises to 20 to 30 μ*M* following oral administration of choline or its precursor, lecithin (*20*). The concentration of choline can increase in response to other situations. In mouse brain, choline levels increase to greater than 100 μmole per kilogram of tissue during hypoglycemic stupor (*21*). In the present study, about 1 m*M* choline was required to induce detectable nicotinic effects in cultured bovine chromaffin cells. If nicotinic receptors in humans have a similar sensitivity to choline, then the increase in the concentration of choline that follows lecithin or choline administration may have subtle effects on systems involving such receptors. For example, neuronal circuits with multiple nicotinic synapses may be especially sensitive to the effects of choline.

These findings may also have implications for the function of a normal cholinergic synapse. The release of one quantum of acetylcholine at the neuromuscular junction results in a local synaptic

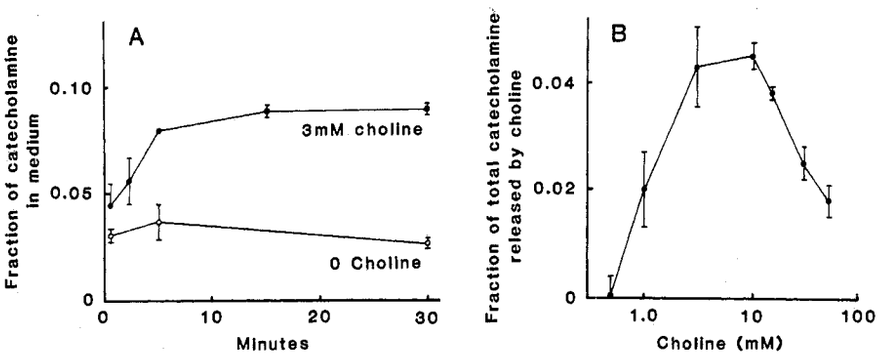

Fig. 1. Choline-induced catecholamine secretion. (A) Time course. Monolayers of chromaffin cells were incubated in 0.5 ml of PSS in the presence or absence of choline chloride (Eastman Kodak). Secretion was halted by transferring the incubation solution to a test tube containing 0.056 ml of 50 percent trichloroacetic acid (TCA). TCA solution (5 percent; 0.5 ml) was immediately added to the monolayers to liberate the remaining catecholamines. Total catecholamine (norepinephrine and epinephrine) in the TCA-containing solutions was measured (*23*) and the fraction of the total catecholamine in the medium was calculated. Each data point is the mean value for three wells. (B) Choline dose-response curve. Cells were incubated for 15 minutes in PSS containing various concentrations of choline chloride. In solutions containing choline at concentrations greater than 3 m*M*, NaCl was reduced to maintain isotonicity. Catecholamines in the medium without added choline (0.020 percent of the total catecholamine) were subtracted from the values shown in (B).

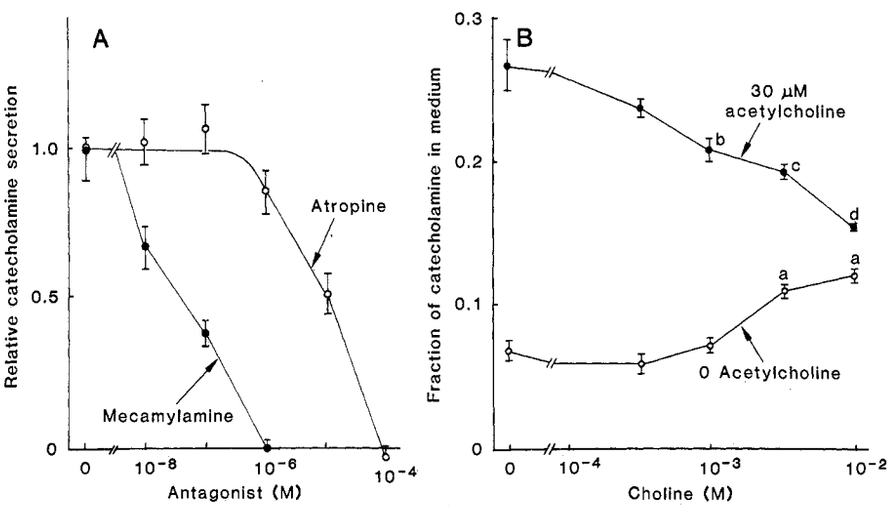

Fig. 2. (A) Effect of cholinergic antagonists on choline-induced secretion. Cells were incubated for 15 minutes in PSS with various concentrations of antagonist. The solution was then aspirated and replaced with solution containing the same concentration of antagonist and 3 m*M* choline. The fraction of the total catecholamine secreted during a 15-minute incubation was then determined. (B) Effect of choline on acetylcholine-induced secretion. Cells were incubated for 15 minutes in various concentrations of choline in the presence or absence of 30 μ*M* acetylcholine. All solutions contained the anticholinesterase physiostigmine sulfate (1 μ*M*). The value indicated by *a* is significant at $P < .01$ compared to the values obtained in the absence of agonist. Values indicated by *b*, *c*, and *d* are significant at $P < .05$, .01, and .001, respectively, compared to the values obtained in the presence of 30 μ*M* acetylcholine and the absence of choline. Each data point in (A) and (B) is the mean value for three wells.

acetylcholine concentration of approximately 0.3 m*M* (*22*). Simultaneous or successive release of multiple quanta is likely to result in even larger concentrations of the hydrolyzed product choline, although neuronal choline uptake and diffusion away from the neuromuscular junction cause uncertainty as to the maximum choline concentrations actually attained. Similar local concentrations of choline may occur at other nicotinic cholinergic synapses. Thus, choline concentrations comparable to those used in the present experiments may occur physiologically and exert effects because of choline's activity as a partial nicotinic agonist.

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15. Maximal catecholamine secretion is induced by 0.1 m*M* acetylcholine. Concentrations of acetylcholine as high as 1 m*M* do not inhibit this secretion. Hence, acetylcholine synthesized in the cultures would not be expected to inhibit the response to 30 μ*M* acetylcholine.
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Multiple Opiate Receptors: Alcohol Selectively Inhibits Binding to Delta Receptors

Abstract. *The addition of ethanol or other aliphatic alcohols to rat brain membranes strongly inhibits binding of enkephalins at concentrations at which little inhibition of opiate alkaloids is seen. Inhibition is reversible, and potency increases with chain length of the alcohol. The results suggest that* δ *receptors are considerably more sensitive to alcohols than* μ *receptors. This is the first demonstration of selective inhibition of one of the postulated classes of opiate receptors by a reagent that is not a ligand for the receptor.*

In recent years numerous studies have suggested the existence of several classes of opiate receptors. Martin and coworkers (*1*), on the basis of pharmacological studies on spinal dogs, concluded that there are three such classes (μ, κ, and σ). Lord *et al.* (*2*) discovered a difference between the receptors that predominate in guinea pig ileum and those in mouse vas deferens. They called them μ, or morphine-preferring, and δ, or enkephalin-preferring, respectively. Receptor binding studies (*2–4*) produced evidence for the same two types of receptors.

The existence of separate classes of receptors would be supported by evidence for the selective inhibition or inactivation of one of the receptor types. Attempts to inactivate or inhibit μ or δ opiate receptors by reagents other than receptor ligands and their derivatives have been unsuccessful. Irreversible inhibitors such as *N*-ethylmaleimide (*5, 6*) and phenoxybenzamine (*7*) and enzymes such as phospholipase A (*8*) and trypsin (*9*) were found to produce equal inactivation of enkephalin and opiate binding. This has necessitated the use of indirect approaches, such as studies of the protection of enkephalin and opiate binding against inhibitors by various receptor ligands (*6, 7*). We now report that ethanol and other aliphatic alcohols selectively inhibit the binding of enkephalin and its stable analogs at concentrations that have little or no effect on the binding of opiate alkaloids. Our results indicate that δ receptors are selectively inhibited.

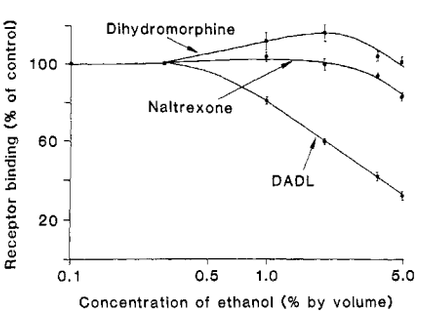

Fig. 1. Effects of ethanol on the binding of ^{3}H-labeled opioids to opiate receptors in rat brain membrane preparations. Duplicate 2-ml samples (0.9 to 1.1 mg of protein per milliliter) in 0.05*M* tris HCl (*p*H 7.4) containing 1 m*M* dipotassium EDTA were incubated with 1 n*M* [^{3}H]dihydromorphine (specific activity, 73.2 Ci/mmole), [^{3}H]naltrexone (8.5 Ci/mmole), and [^{3}H]DADL (31.0 Ci/mmole). To assess specific binding, samples were incubated in the presence or absence of 1 μ*M* unlabeled ligand. Incubations were followed by cooling in an ice water bath for 10 minutes before filtration. The values represent the means ± standard errors for at least three experiments.

Membrane preparations were made from rat brain, toad brain, and neuroblastoma cells (N4TG1) (*10*). Binding studies were carried out with [^{3}H]naltrexone, [^{3}H]dihydromorphine, and tritiated D-Ala^2-D-Leu^5-enkephalin (DADL). The alcohol was added just before addition of the labeled ligand and incubation was carried out at 37°C for 15 minutes in the presence or absence of a 1000-fold excess of unlabeled ligand. Filtration and scintillation counting were done as previously described (*10*).

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