cording to published methods [J. A. Herd, W. H. Morse, R. T. Kelleher, L. G. Jones, *Am. J. Physiol.* **27**, 24 (1969)]. While the monkey was under halothane anesthesia and in aseptic conditions, one end of a polyvinyl chloride catheter (inside diameter, 0.38 mm; outside diameter, 0.76 mm) was passed by way of the left or right external jugular vein into the superior vena cava at the level of the right atrium. The distal end of the catheter was passed subcutaneously and exited through the skin in the middle of the monkey's back. Each monkey wore a leather jacket at all times to protect the catheter. Catheters were flushed daily with 0.9 percent saline solution and were sealed with stainless steel obturators when not in use.

6. Cocaine HCl was dissolved in 0.9 percent saline solution. The volume of each injection was 0.20 ml, infused over 200 msec. An amber feedback light was presented for 1 second before each injection. The doses (as the salt) per injection were as follows: 10 μg per kilogram of body weight (monkey S-146), 30 μg/kg (monkeys S-153 and S-154), and 100 μg/kg (monkey S-332). These doses were selected on the basis of preliminary observations and maintained responding throughout each session for individual monkeys.
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8. The doses of cocaine that were infused before each session equaled the average cumulative doses that were self-administered during the last three sessions before saline substitution. These doses were 0.55 mg per kilogram of body weight (monkey S-154) and 1.93 mg/kg (monkey S-332). Cocaine solutions were infused through the catheter in a volume of 1.0 ml/kg over a 2-minute period. The catheter was then flushed with saline, and the session began 5 minutes later.
9. G. B. Kish [in *Operant Behavior: Areas of Research and Application*, W. K. Honig, Ed. (Appleton-Century-Crofts, New York, 1966)] reported that a change in exteroceptive (for example, visual) stimulation can, under some conditions, maintain responding; R. T. Hill [in *Amphetamines and Related Compounds*, E. Costa and S. Garattini, Eds. (Raven, New York, 1970)] and T. W. Robbins [*Psychopharmacologia* **45**, 103 (1975)] reported that the effectiveness of an exteroceptive-stimulus change in maintaining responding can, under some conditions, be enhanced by the presence of psychomotor-stimulant drugs. These effects appear to have contributed minimally to the present results.
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## Norepinephrine Inhibits Calcium-Dependent Potentials in Rat Sympathetic Neurons

Abstract. *Norepinephrine reversibly antagonizes three calcium-dependent potentials recorded from rat postganglionic neurons. Norepinephrine inhibits the development of a shoulder on the action potential, the magnitude of the hyperpolarizing afterpotential, and the rate of rise and amplitude of the calcium spike. The action of norepinephrine is antagonized by the α-adrenergic antagonist phentolamine, but not by MJ 1999, a β-adrenergic antagonist. These results suggest that activation of an α-adrenergic receptor may antagonize a voltage-sensitive calcium current.*

Ion conductance mechanisms enable neurons to regulate membrane potential and alter intracellular ion concentrations. Voltage-sensitive calcium conductances, $g\mathrm{Ca}_{(v)}$, and calcium-sensitive potassium conductances, $g\mathrm{K}_{(Ca)}$, have been observed in neurons from both invertebrate and vertebrate species (*1*). These conductances can play important physiological roles, including the control of repetitive firing of neurons, pacemaker activity, and neurotransmitter release (*2*). A related event is the hyperpolarizing afterpotential (HAP) of the rat postganglionic neuron. Yarowsky and McAfee (*3*) have shown that the HAP results from an increased $g\mathrm{K}$ which is largely dependent upon extracellular $Ca^{2+}$. In addition, postganglionic neurons support a regenerative $Ca^{2+}$ action potential that is insensitive to tetrodotoxin (TTX), the $Na^+$ conductance antagonist. Analysis of the $Ca^{2+}$ spike and HAP (*3*) indicates that these neurons possess both $g\mathrm{Ca}_{(v)}$ and $g\mathrm{K}_{(Ca)}$ and suggests that inward $Ca^{2+}$ current triggers part of the HAP (*4*). We now report that norepinephrine reversibly antagonizes both the HAP and the $Ca^{2+}$ spike, and propose that norepinephrine acts through an α-adrenergic receptor to inhibit $Ca^{2+}$ current.

Superior cervical sympathetic ganglia isolated from mature Sprague-Dawley rats (150 to 250 g) were desheathed and perfused with oxygenated Locke solution for up to 12 hours in vitro (24° to 26°C). Intracellular recordings were made with glass microelectrodes from postganglionic cells visualized through a compound microscope. Membrane potential was controlled by current passed through the microelectrode. The composition of the perfusion medium was altered as indicated to apply drugs or to change extracellular ion concentrations (*3*, *5*).

We have identified three voltage responses in postganglionic neurons which are $Ca^{2+}$-dependent. Two, the HAP and a "shoulder," are seen during discharge in unmodified Locke solution. In this case, a single, brief depolarizing current pulse (100 to 1000 pA, 4 to 15 msec), applied through the recording microelectrode, resulted in action potentials that began with a rapid depolarization followed by a less rapid repolarization having a distinct inflection or shoulder (Fig. 1); the repolarization overshot the resting potential by 12 mV, producing a HAP lasting 300 msec (Fig. 1). This pattern was reproduced in all 75 cells studied. Both the shoulder and the HAP decreased in magnitude as extracellular $Ca^{2+}$ concentration, $[Ca^{2+}]_0$, was reduced (Fig. 1). Divalent cobalt (0.5 to 3.0 m*M*), a $Ca^{2+}$ antagonist in this and other preparations (*1*, *3*), also reduced the shoulder and the HAP with little or no change in the rising phase or duration of the action potential. These $Ca^{2+}$-dependent potentials appeared to be independent of the voltage-sensitive $K^+$ conductance [delayed rectification, $g\mathrm{K}_{(v)}$], inasmuch as tetraethylammonium (TEA), an antagonist of $g\mathrm{K}_{(v)}$, augmented both the action potential shoulder and the duration of the HAP. When TEA was used, the enlarged shoulder and HAP remained sensitive to low $[Ca^{2+}]_0$ or 3 m*M* $Co^{2+}$.

The third $Ca^{2+}$-dependent potential is the regenerative discharge produced by depolarization of postganglionic neurons bathed in Locke solution containing TTX (1 μ*M*) and TEA (5 m*M*) (Fig. 1). Yarowsky and McAfee (*3*) concluded that this action potential was a $Ca^{2+}$ spike because its amplitude was proportional to $[Ca^{2+}]_0$ and antagonized by $Co^{2+}$. This spike was followed by a HAP with characteristics similar to the HAP in normal Locke solution.

Norepinephrine (10 μ*M*) reversibly inhibited all of these three $Ca^{2+}$-dependent potentials. It reduced the action potential shoulder and the magnitude of the HAP in all neurons studied ($N = 25$) (Fig. 1); the average attenuation of the HAP was about 40 percent. The membrane resistance decrease associated with the HAP was also reduced by norepinephrine. These effects occurred after less than 1 minute of exposure to norepinephrine, did not desensitize over a 15-minute period, and were reversed within 10 minutes of washing. A minimum detectable effect was produced at a concentration of 0.1 to 0.3 μ*M*. A 10-μ*M* concentration sometimes produced a slight hyperpolarization (0.5 to 4.0 mV) of resting membrane potential which was not accompanied by a detectable change in resting input resistance (*6*). The effects of norepinephrine on the action potential and the HAP were seen even when the


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control resting potential was reestablished by passage of constant current through the recording electrode.

In order to ascertain the effect of norepinephrine on calcium spikes, some neurons were perfused with Locke solution containing TTX (1 $\mu M$) and TEA (5 m$M$). Norepinephrine (10 $\mu M$) reversibly diminished both the rate of rise and amplitude of the $Ca^{2+}$ spike and the amplitude of its associated HAP (Fig. 1).

Dopamine and isoproterenol (10 $\mu M$) produced effects qualitatively similar to those of norepinephrine but were less efficacious. Phentolamine (10 $\mu M$), an $\alpha$-adrenergic antagonist, inhibited the actions of norepinephrine (Fig. 1). In contrast, MJ 1999 (10 $\mu M$), a $\beta$-adrenergic antagonist, was without effect. These findings suggest that norepinephrine acts through an $\alpha$-adrenergic receptor.

While the action of norepinephrine on the three $Ca^{2+}$-dependent potentials was mimicked by low $[Ca^{2+}]_0$ or $Co^{2+}$ (3 m$M$), the responses that remained present in low $[Ca^{2+}]_0$ or $Co^{2+}$ were not further antagonized by norepinephrine.

In summary, we have observed that norepinephrine specifically inhibits the $Ca^{2+}$-dependent portions of the action potential, the HAP, and the $Ca^{2+}$ spike. A reasonable hypothesis to account for these observations is that norepinephrine inhibits an inward $Ca^{2+}$ current. Previous studies (*3*) suggest from indirect evidence that the $Ca^{2+}$ spike is generated solely by an increase in $gCa_{(v)}$, which in turn triggers an increase in $gK_{(Ca)}$, resulting in a HAP. A voltage-sensitive $gK_{(v)}$ also contributes to the HAP. If intense enough, a voltage-sensitive $Ca^{2+}$ current could contribute sufficiently to the currents during the normal action potential to account for the $Ca^{2+}$-dependent shoulder during repolarization. This has been observed in invertebrate neurons (*7*). Alternatively, the kinetics of $g$Na and $g$K during the spike may be altered by $Ca^{2+}$. When achieved, voltage-clamp analysis of these neurons will provide more direct evidence of ionic conductance mechanisms. However, antidromic stimulation results in the accumulation of $^{45}Ca$ in the rat ganglion (*8*). Others have observed that norepinephrine inhibits $Ca^{2+}$ spikes in bullfrog sympathetic ganglia (*9*) and the action potential shoulder in cultured cells from the chick dorsal root ganglion (*10*). Such an action of norepinephrine has important implications. Does the $\alpha$ receptor directly affect the $Ca^{2+}$ channel or does it have a more indirect action? Norepinephrine could act indirectly by increasing intraneuronal $Ca^{2+}$ as it does in some smooth muscle cells (*11*), and small increases in intracellular $Ca^{2+}$ could thus inhibit $gCa_{(v)}$ as it does in certain invertebrate neurons (*12*). Finally, catecholamines antagonize synaptic transmission in mammalian sympathetic ganglia (*13*) by an action at a presynaptic site through an $\alpha$ receptor and reduction in neurotransmitter release (*6, 14*). Perhaps this presynaptic action of catecholamines is to reduce $Ca^{2+}$ influx into the terminal, which results in reduced quantal content. Whatever the mechanisms by which catecholamines antagonize $Ca^{2+}$-dependent processes, it is clear that these actions will greatly influence neuronal function.

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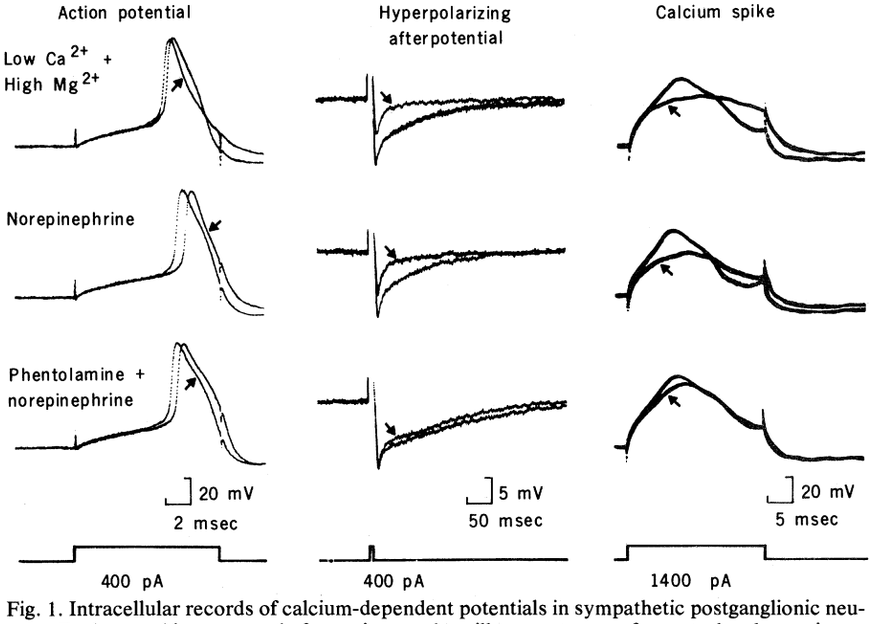


Fig. 1. Intracellular records of calcium-dependent potentials in sympathetic postganglionic neurons. Each record is composed of superimposed oscilloscope traces of a control and experimental (arrow) response. These voltage responses were initiated by depolarizing currents passed through the microelectrode (rectangular pulses at the bottom). The left vertical column contains records obtained at a rapid sweep to best illustrate the action potential "shoulder" in one cell. The middle column records were obtained from the same cell at a slower sweep to best illustrate the HAP. Records of $Ca^{2+}$ spikes are presented in the right column from another cell incubated in TTX (1 $\mu M$) and TEA (5 m$M$). In the top horizontal row, the control traces were made just before, and the experimental traces (arrow) were made 3 minutes after the usual divalent cation composition was altered to 0.6 m$M$ $CaCl_2$, 10 m$M$ $MgCl_2$. In the middle row, the usual ionic composition was maintained, but the experimental traces (arrow) were generated 3 minutes after exposure to 10 $\mu M$ norepinephrine. In the bottom row, the experiment of row 2 was repeated after 30 minutes of incubation in 10 $\mu M$ phentolamine, an $\alpha$-adrenergic antagonist. The low $Ca^{2+}$, high $Mg^{2+}$ condition antagonized the action potential shoulder, HAP, and $Ca^{2+}$ spike (top row). Norepinephrine mimicked the effect of low $Ca^{2+}$ (middle row) except in the presence of phentolamine (bottom row). Long, depolarizing pulses were used to facilitate superimposition of the responses. Similar results were obtained when brief pulses were used that did not outlast the rising phase of the action potential or $Ca^{2+}$ spike. The resting potential for both cells was about −55 mv.

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4. A $gK_{(Ca)}$ mechanism in rat postganglionic neurons is suggested by the following observations (*3*): (i) the membrane conductance increases during the HAP; (ii) the HAP amplitude and reversal potential are dependent upon −59 log $[K^+]_0$; (iii) $Co^{2+}$, $Mn^{2+}$, $Cd^{2+}$, and low $Ca^{2+}$ reduce the HAP conductance and amplitude; and (iv) the HAP is augmented during repetitive firing. A $gCa_{(v)}$ mechanism is suggested by the following: (i) postganglionic neurons generate a voltage-dependent regenerative spike when $gNa_{(v)}$ and $gK_{(v)}$ are blocked by TTX and TEA; (ii) this spike is $Ca^{2+}$-dependent and present even in isotonic $CaCl_2$; (iii) this $Ca^{2+}$-dependent spike occurs with an increase in membrane conductance; and (iv) the magnitude of the HAP is directly proportional to the amplitude and duration of the $Ca^{2+}$-dependent spike.
5. The usual composition of Locke solution was as follows: NaCl, 136 m$M$; KCl, 5.6 m$M$; $CaCl_2$, 2.2 m$M$; $MgCl_2$, 1.2 m$M$; $NaH_2PO_4$, 1.2 m$M$; $NaHCO_3$, 20 m$M$; and dextrose, 8.3 m$M$. This solution was equilibrated with a mixture of 95 percent $O_2$ and 5 percent $CO_2$ throughout the experiment and had a $p$H of 7.2 at the temperature of the experiment (24°C). Tetrodotoxin (1 $\mu M$) and tetraethylammonium chloride (5 m$M$) were added to this Locke solution in the $Ca^{2+}$ spike experiments. Low $Ca^{2+}$ experiments were done in high (10 m$M$) $MgCl_2$ to counteract charge screening effects. High $MgCl_2$ alone acted as a very weak antagonist of $Ca^{2+}$-dependent potentials. A cold, aqueous stock solution of *l*-norepinephrine hydrochloride was diluted 500-fold into the Locke solution just before perfusion. Regitine (phentolamine methylsulfonate) was used to antagonize $\alpha$-adrenergic responses. Standard

microelectrode techniques were employed as described (3).
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## Brain Stimulation Reinforcement: Implications of an Electrode Artifact

Beninger *et al.* (*1*) concluded that the lever pressing of rats reinforced directly with electrical brain stimulation reinforcement (BSR) (i) does not show a sharp decline when BSR is terminated and (ii) is as easily brought under the control of a lean intermittent schedule of BSR as it is under a schedule of food reinforcement. These conclusions, which I dispute, conflict with numerous empirical reports (*2*) and suggest that three major theoretical accounts (*3, 4*) aimed at understanding these "anomalies" of BSR are unnecessary.

The authors' conclusions are partly based on their inability to replicate my finding (*5*) that if a response-dependent BSR was preceded by a brief warning signal (a cue light) rather than immediately following the response, (i) the acquisition of control by an intermittent schedule [such as fixed ratio (FR), 200; variable interval (VI), 2 minutes; or fixed interval (FI), 3 minutes] was facilitated and (ii) a higher response rate was maintained on time-based schedules. I have found that the effect of the warning signal in my experiment depends upon an artifact.

The artifact is caused by an electrode and plug assembly (Plastic Products, Inc.) that has the potential for movement-produced electrical discontinuity and graded conductance change at the interface. As a result, preceding the BSR with a brief warning signal imposed a chain schedule: the signal functioned as a discriminative stimulus ($S^D$) for the animal's postural adjustment which, because of the artifact, allowed an increase in conductance and charge, and consequently a greater magnitude of reinforcement. This was shown by data for four rats that acquired lever pressing reinforced by signaled BSR in both components of a multiple VI 1-minute, VI 1-minute schedule. A constant voltage stimulator was employed. After behavior stabilized, BSR was signaled in one component and unsignaled in the other. Substantially more current passed when the BSR was signaled rather than unsignaled (Fig. 1). The asymptotic response rate was 8 to 20 percent higher in the component associated with signaled BSR. Reversal of the component stimuli with respect to signaling of BSR produced the same result. When a new "captive" collar (Plastic Products, Inc.) was substituted for the older model plug and collar, current flow for signaled and unsignaled BSR was nearly the same and response rate for the two schedule components did not differ. The ability of the signaled BSR to maintain higher response rates is thus an artifact of the older model plug assembly (*6*). Furthermore, without the artifact making the signal an $S^D$ in a chain schedule, the signaling procedure is as poor as or worse than simple response-contingent BSR (*7*) in bringing about lever pressing on an intermittent schedule. The efficacy of a chain schedule in establishing schedule performance with BSR was first shown by Pliskoff *et al.* (*8*) with multiple trains of stimulation per reinforcement. My own experiment (*5*) shows that a single train of BSR can serve as reinforcement in the terminal link of an intermittent chain schedule.

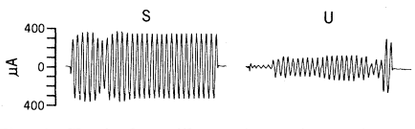


Fig. 1. Typical oscillograph tracings of current passed as a function of whether the BSR was signaled (*S*) or unsignaled (*U*). The signal was a 7-W cue light above the lever. Response-contingent signaled and unsignaled stimulation was presented in two components (200- or 1100-Hz tones) of a multiple VI 1-minute, VI 1-minute schedule. Components changed with a probability of 0.3 after a BSR. The stimulation was 60-Hz sinusoidal, constant voltage; values for the four subjects varied from at 1.5 to 2.5 V. The electrodes were bipolar platinum twisted pairs.

Beninger *et al.* (*1*) claim to show in their third experiment that performance typical of that with food reinforcement is produced with simple response-contingent BSR and that a special procedure such as chaining is therefore unnecessary. But their response rates of 5 per minute are exceedingly low for a random interval (RI) 45-second schedule. Even the highest rates reported (10 per minute) are considerably lower than the 20 per minute found in my experiment with a substantially leaner VI 2-minute schedule. Beninger *et al.* reported rapidly declining response rates as the RI value was increased. Perhaps this discouraged attempts to maintain responding on such schedules as FR 200, VI 2 minutes, and FI 3 minutes, as shown in experiments in which a chaining procedure was used (*5, 8*). Only evidence (with cumulative records) of such schedule performance should be taken in support of the assertion that chaining is unnecessary.

Imposing a chain schedule with BSR prevents the occurrence of the rapid extinction effect (REE) (*9*)—a sharp drop-off in responding when extinction is programmed after continuous reinforcement (CRF) training—during the transition from CRF to the intermittent schedule. The REE is peculiar to BSR because BSR is ordinarily applied in the last and only link of a chain schedule, whereas food reinforcement requires many links in a chain of consumatory behavior. In 1934, Skinner (*10*) asserted in the language of the day that "in a chain of reflexes not ultimately reinforced, only the members actually elicited undergo extinction." In other words, responses after the break are protected from extinction because $S^D$'s for them are not presented. On the other hand, $S^D$'s before the break are presented and their responses are extinguished. Consequently, the REE reflects the breakage of the all-important last and only link of a one-link chain. When extinction after CRF with food reinforcement occurs, many links after the break are "not elicited" and the operant is thereby better protected from extinction. Similarly, the response decrement in extinction after CRF training is slower when a foodlike chain is arranged than when BSR is directly contingent on the operant (*11*). Almost all successful attempts to maintain behavior on an intermittent schedule of BSR have employed a chain that protects the operant


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