2/3 stage (Wilcoxon MPSRT: $T = 1$, $N = 8$ pairs, $P < .02$; (Fig. 1B). Among this set of lizards dominants lost their social advantage after loss of one-third of their tails but regained their status after the other pair members (new dominants) lost two-thirds of their tails. In fact, social polarity of these pairs at the 2/3 stage was not significantly different from the 0 stage (Wilcoxon MPSRT: $T = 7$, $N = 8$ pairs, $P > .10$) (Fig. 1B).

In nature, nutrition, age, past social experience, site of encounter, endocrine environment, and so on, can affect social status. By controlling many of these factors, we were able to observe the effect of tail loss on social relationships (*16*). A juvenile *U. stansburiana* that loses its tail and becomes subordinate to nearby conspecifics, may be unable to secure a high-quality home range (*11*) and in turn be subject to an increased risk of death (*17*). Tail autotomy is probably a predator defense of last resort.

The nine pairs of lizards for which a reversal in social polarity was observed at the 1/3 stage had initially (0 stage) been closer to social parity than the other 21 pairs (Mann-Whitney U test: $z = 2.29$, $N = 21,9$, $P < .05$) (Fig. 2). This difference alone, however, does not account for their reversal in status. The decrease in social polarity from the 0 to the 1/3 stage for these pairs was also significantly greater than that for the others (Mann-Whitney U test, $z = 4.01$, $N = 21, 9$, $P < .001$).

Why these nine pairs were initially closer to social parity is not related to sex or body size. The original dominants (0 stage) were slightly heavier than the original subordinates (Wilcoxon MPSRT: $T = 88$, $N = 28$ pairs, $P < .01$), but the weight disparity was similar for all 30 pairs. Total tail lengths and tail-body ratios of the nine pairs were not different from the others. In short, we could find no morphological differences to explain why some pairs reversed status at the 1/3 stage, and some did not.

It was only when two-thirds of the tail was lost that the lizards in the first experiment showed a social effect (Fig. 1A). Tail replacement (stump growth plus regeneration) is fastest for tails broken most proximally (*7*). Although this fast basal regeneration is a proximate physiological response (*18*), an ultimate benefit is the rapid return of the tail to a length sufficient to restore the social status of the lizard after significant loss of its tail.

These experiments were not designed to separate the effect of loss of total body length from the complete syndrome of tail loss (that is, major body wound, loss of organ of balance, loss of stored energy, and so on). The deficiencies other than loss of length may also contribute to loss of social status.

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References and Notes

1. K. P. Schmidt and R. F. Inger, *Living Reptiles of the World* (Hanover House, Garden City, N.Y., 1957); C. C. Carpenter, *Proc. Calif. Acad. Sci.* **34**, 329 (1966); R. Etheridge, *Copeia* **1967**, 699 (1967); R. E. Ballinger, *Herpetologica* **29**, 65 (1973); L. J. Vitt and R. D. Ohmart, *ibid.* **31**, 56 (1975); R. E. Ballinger, J. W. Nietfeldt, J. J. Krupa, *ibid.* **35**, 114 (1979).
2. L. J. Vitt, J. D. Congdon, N. A. Dickson, *Ecology* **58**, 326 (1977).
3. J. D. Congdon, L. J. Vitt, W. W. King, *Science* **184**, 1379 (1974).
4. R. G. Zweifel and C. H. Lowe, *Am. Mus. Novit. 2247* (1966), pp. 1–57; R. E. Ballinger and D. W. Tinkle, *J. Herpetol.* **13**, 374 (1979).
5. M. Smyth, *Aust. J. Zool.* **22**, 135 (1974); V. C. Maiorana, *Nature (London)* **265**, 533 (1977); B. E. Dial and L. C. Fitzpatrick, *Oecologia (Berlin)* **51**, 310 (1981).
6. D. R. Clark, *J. Exp. Zool.* **176**, 295 (1971).
7. D. W. Tinkle, *Misc. Publ. Mus. Zool. Univ. Mich. 132* (1967).
8. C. C. Carpenter and G. Grubitz, *Southwest. Nat.* **5**, 123 (1960); B. H. Brattstrom, *Am. Zool.* **14**, 35 (1974); M. F. Brackin, *Herpetologica* **34**, 185 (1978).
9. A. S. Rand, *Proc. U.S. Nat. Mus.* **122**, 1 (1967); D. A. Boag, *Oecologia (Berlin)* **12**, 1 (1973); K. H. Berry, *Univ. Calif. Berkeley Publ. Zool. 101* (1974); D. E. Ruby, *Copeia* **1978**, 430 (1978); J. A. Stamps, *Behaviour* **66**, 1 (1978); J. J. Talbot, *Copeia* **1979**, 472 (1979); D. E. Ruby, in *Natural Selection and Social Behavior*, R. D. Alexander and D. W. Tinkle, Eds. (Chiron, New York, 1981), p. 96.
10. T. H. Fleming and R. S. Hooker, *Ecology* **56**, 1243 (1975); R. L. Trivers, *Evolution* **30**, 253 (1976); J. A. Stamps, *Ecology* **58**, 349 (1977).
11. S. F. Fox, E. Rose, R. Myers, *Ecology* **62**, 888 (1981).
12. K. H. Berry, *Univ. Calif. Berkeley Publ. Zool. 101* (1974).
13. First-year lizards were collected from Winkler County, Texas, in early September 1980. Fifteen pairs of each sex were matched in size by snout-vent length (within 2.0 mm) and by total length (within 3.0 mm).
14. Both sexes show aggression and equivalent agonistic behavior patterns (*11*). Lateral displays, supplants, bites and licks, and superimpositions were weighted 1.0. Push-ups, because of their high rate of delivery, apparently low energetic cost, and frequent nondirected delivery (that is, assertion bobs), were weighted 0.5. The single submissive pattern observed, ventral flattening (*17*), was weighted −1.0. The agonistic score for each lizard was computed as the sum of weighted frequencies of agonistic behavior patterns.
15. The tails were autotomized by the lizards when pinched firmly between the experimenter's fingers, simulating the bite of a predator. *U. stansburiana* does not drop its tail without such stimulus.
16. Body growth during tail regeneration is often slowed in lizards (*2, 4*). Under the conditions of captivity and short timespan of these experiments, however, growth in snout-vent length within pairs was equivalent; differences in snout-vent length within pairs did not significantly change over the experimental interval (Wilcoxon MPSRT: 0 to 2/3 stage, $T = 56$, $N = 16$ nonzero pairs, $P > .05$; 1/3 to 2/3 stage, $T = 6$, $N = 7$ nonzero pairs, $P > .05$).
17. S. F. Fox, *Ecology* **59**, 834 (1978).
18. L. E. Iten and S. V. Bryant, *J. Exp. Zool.* **196**, 293 (1976).
19. We thank S. Bontrager, C. Bloom, and E. Shoemaker for logistical aid. Supported in part by NSF grant DEB-78-07156 to S.F.F.

8 March 1982; revised 21 June 1982

Cotransmitters in the Motor Nerves of the Guinea Pig Vas Deferens: Electrophysiological Evidence

Abstract. *The contractile response of the guinea pig vas deferens to motor nerve stimulation is biphasic. The first phase is antagonized by the specific adenosine triphosphate–receptor antagonist arylazido aminoproprionyl adenosine triphosphate ($ANAPP_3$), and the second by the α-receptor antagonist prazosin. The underlying electrical event, the excitatory junction potential, is also blocked by $ANAPP_3$, but not by prazosin.*

The contractile response of the guinea pig vas deferens to a single pulse, or to short trains of stimuli, is biphasic; the second phase (phase II) is blocked by α_1-receptor antagonists and is therefore assumed to be adrenergic, while the initial twitch response (phase I) is resistant to adrenergic antagonists (*1, 2*). Our laboratory recently showed that phase I is specifically antagonized by arylazido aminoproprionyl adenosine triphosphate ($ANAPP_3$), a photoaffinity label and structural analog of adenosine triphosphate (ATP) which has been shown to be a specific antagonist of the P_2 class of purine receptors in this tissue (*3, 4*). On the basis of these findings, it has been proposed that the biphasic nature of the contractile response is a result of the action of cotransmitters: ATP or a related purine mediating phase I through P_2-receptors and norepinephrine mediating phase II through α_1-receptors.

Blakeley *et al.* (*5*) recently demonstrated in the guinea pig vas deferens that the action potential and phase I of the contractile response to motor nerve stimulation are blocked by the calcium channel blocker nifedipine, while phase II is largely unaffected. This implies that phase I is dependent on the summation of excitatory junction potentials (EJP's) to threshold and the firing of action potentials producing the initial switch. Since $ANAPP_3$ is able to antagonize phase I specifically, and since phase I is dependent on the summation of EJP's, we investigated the effect of $ANAPP_3$ on EJP's in this muscle.

Intracellular microelectrodes filled

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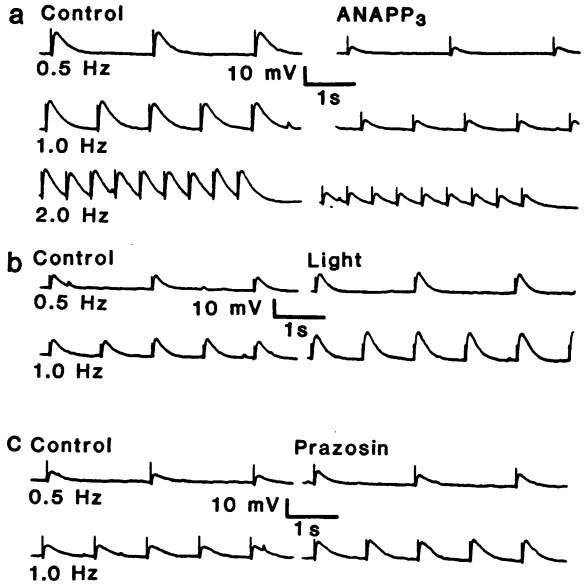

Fig. 1. (a) Excitatory junction potentials recorded from guinea pig vas deferens at stimulation frequencies of 0.5, 1.0, and 2.0 Hz. Responses are from cells in the same muscle recorded before and after treatment with the P_2-receptor antagonist $ANAPP_3$ (resting membrane potentials, 58 mV for control cell and 62 mV for $ANAPP_3$-treated cell. The vertical line preceding each EJP is the stimulus artifact. (b) Excitatory junction potentials recorded from cells in the same muscle before and after treatment with light only. Note that, after $ANAPP_3$ treatment, EJP's are substantially reduced in size, but are not reduced by light alone. (c) Continuous recording, from the same cell (resting membrane potential, 66 mV), of EJP's at 0.5 and 1.0 Hz. Fifteen minutes after the introduction of $10^{-6}M$ prazosin, EJP's increased in size by about 40 percent compared with control EJP's.

with $3M$ KCl (20 to 60 megohms) were used to record EJP's evoked by field stimulation from two platinum ring electrodes around the prostatic end of the vas deferens. Pulse width was 0.5 msec at a supramaximal voltage. After control EJP's were recorded from several cells the vas deferens was removed from the recording chamber and mounted in a continuously suffused organ bath for treatment with $ANAPP_3$ (*4*). The tissue was photolyzed for 20 minutes with a (DVY, tungsten halogen projector lamp 650 W, 3400 K) in the presence of $10^{-4}M$ $ANAPP_3$. This treatment produces an irreversible antagonism of P_2-receptor–mediated responses. The tissue was returned to the recording chamber and washed with fresh Krebs solution for at least 15 minutes to remove $ANAPP_3$. EJP's were then recorded from several more cells. Tissues treated with light for the same period without any $ANAPP_3$ served as "light controls." In all experiments recordings were made for only 60 minutes after the tissue was returned to the recording chamber.

Figure 1a shows EJP's recorded before and after the tissue was treated with $ANAPP_3$. At 0.5 Hz the average size of the EJP's was 8.6 ± 0.48 mV (53 cells from 14 muscles) before $ANAPP_3$ and only 2.5 ± 0.26 mV (38 cells from 9 muscles) afterward. Even when $ANAPP_3$-treated tissues were stimulated at 5 Hz the average size of the EJP's was only 6.6 ± 0.67 mV (ten cells from five muscles). The EJP's recorded from cells of tissues used as light controls showed no significant change from the untreated group, having an average size of 9.1 ± 0.53 mV (29 cells from 9 muscles) at 0.5 Hz. The average resting membrane potential of cells from the $ANAPP_3$-treated and light control groups was not significantly different from that of cells from untreated muscles—62.1 + 0.1 mV (50 cells from 14 muscles).

The biphasic nature of the contractile response of the guinea pig vas deferens to field stimulation is illustrated in Fig. 2. Treatment with $ANAPP_3$ greatly reduced phase I of the response but not phase II. In contrast, $10^{-6}M$ prazosin reduced phase II of the contractile response. The ability of prazosin to reduce phase II selectively implies that the neurotransmission process involves α_1-receptors. We also investigated the effect of $10^{-6}M$ prazosin on EJP's. If prazosin was introduced during continuous recording from a single cell, then there was considerable enhancement of EJP's (Fig. 1c). In seven such continuous recordings the average increase in EJP size was 30 ± 4.9 percent after 10 minutes in $10^{-6}M$ prazosin. It seems that, under these conditions, prazosin, although relatively selective for α_1-postsynaptic receptors, may also slightly antagonize α_2-presynaptic receptors. If norepinephrine released by nerve stimulation normally activates these presynaptic receptors to reduce transmitter release, then the presence of prazosin could inhibit this negative feedback process and increase transmitter release from the nerves, resulting in larger EJP's. This effect of $10^{-6}M$ prazosin was also reported by Blakeley *et al.* (*5*). Since the α_1-receptor antagonist produces an increase in EJP's at a dose that abolished phase II of the contraction, it appears that phase II is not dependent on membrane depolarization. This process may be similar to the α_1-receptor–mediated contraction in arterioles, which recently was shown to be independent of membrane depolarization (*6*).

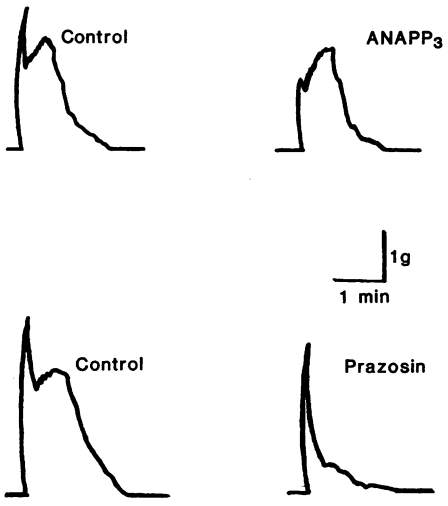

Fig. 2. Contractile responses of the guinea pig vas deferens stimulated at 16 Hz (0.5-msec pulses at supramaximal voltage) for 30 seconds. Tension was recorded isometrically in a continuously suffused organ bath, and field stimulation was by two platinum ring electrodes (*4*). Treatment with $ANAPP_3$ reduced phase I of the contraction while $10^{-6}M$ prazosin reduced phase II. For each drug treatment, the control response of the tissue is shown on the left.

The ability of $ANAPP_3$ to reduce EJP's is unlikely to be due to a nonspecific or neurotoxic effect of the compound, since phase II of the response remains intact (Fig. 2). Preliminary data indicate that $ANAPP_3$ treatment does not alter summation of EJP's or the firing of an action potential once the EJP's summate to threshold, but since the magnitude of EJP's is greatly reduced, threshold is not reached until higher than normal stimulation frequencies are provided.

More detailed studies of the contractile responses of the guinea pig, rat, and rabbit vas deferens (*4*, *7*) also support the view that $ANAPP_3$ is a specific antagonist of phase I of the contractile response and of P_2-mediated responses.

From these results, we propose that, upon stimulation, ATP (or a related purine) is released from motor nerves to produce EJP's, which summate to fire action potentials, which in turn cause phase I of the contractile response. Norepinephrine is also released from these nerves, producing a slower contracture that is independent of membrane depolarization.

The following experimental evidence supports the above hypothesis:

1) Norepinephrine and ATP are

stored together in nerve terminal vesicles of sympathetic nerves (*8*).

2) Norepinephrine and ATP, when added exogenously to the vas deferens, produce contractions that are specifically antagonized by α_1- and P_2-receptor antagonists, respectively.

3) Stimulation of the motor nerves produces a biphasic contraction, and phases I and II are preferentially antagonized by the same α_1- and P_2-receptor antagonists.

4) Reserpine treatment does not abolish EJP's in the guinea pig vas deferens (*9*).

5) Adrenergic antagonists such as phentolamine, phenoxybenzamine, and prazosin readily antagonize phase II but are relatively ineffective in reducing EJP's at similar doses (*10*).

6) The P_2-receptor antagonist ANAPP$_3$ can preferentially block phase I of the contraction and the EJP's.

7) Since EJP's are enhanced by prazosin and blocked by ANAPP$_3$, it would seem unlikely that the two transmitters could interact in this fashion if they were released from different nerve populations. Furthermore, agents that selectively damage sympathetic nerves (such as 6-hydroxydopamine) can inhibit both phase I and phase II of the contractile response (*4*), indicating that both depend on release of transmitter from adrenergic neurons.

8) Finally, ATP and norepinephrine are synergistic in producing contraction when added exogenously, and exogenous ATP enhances contractile responses to nerve stimulation (*11*).

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References and Notes

1. N. Ambache and Zar M. Aboo, *J. Physiol. (London)* **216**, 359 (1971).
2. J. C. McGrath, *ibid.* **283**, 23 (1978).
3. G. K. Hogaboom, J. P. O'Donnell, J. S. Fedan, *Science* **208**, 1273 (1980).
4. J. S. Fedan, G. K. Hogaboom, J. P. O'Donnell, J. E. Colby, D. P. Westfall, *Eur. J. Pharmacol.* **69**, 41 (1981).
5. A. G. H. Blakeley, D. A. Brown, T. C. Cunnane, A. M. French, J. C. McGrath, N. C. Scott, *Nature (London)* **294**, 759 (1981).
6. G. D. S. Hirst and T. C. Neild, *ibid.* **283**, 767 (1980).
7. P. Sneddon, D. P. Westfall, J. Colby, J. S. Fedan, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 1720 (1982).
8. K. B. Helle, H. Langercrantz, L. Stjarne, *Acta Physiol. Scand.* **81**, 565 (1971).
9. G. Burnstock, M. E. Hollman, H. Kuriyama, *J. Physiol. (London)* **172**, 31 (1964).
10. G. Burnstock and M. E. Hollman, *Br. J. Pharmacol.* **23**, 600 (1964).
11. T. Kazic and D. Milosavljevic, *ibid.* **71**, 93 (1980).

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20 July 1982; revised 25 August 1982

Iron-Containing Cells in the Honey Bee (*Apis mellifera*)

Abstract. *Honey bees are sensitive to earth strength magnetic fields and are reported to contain magnetite (Fe_3O_4) in their abdomens. We report bands of cells around each abdominal segment that contain numerous electron-opaque, iron-containing granules. The iron is principally in the form of hydrous iron oxides.*

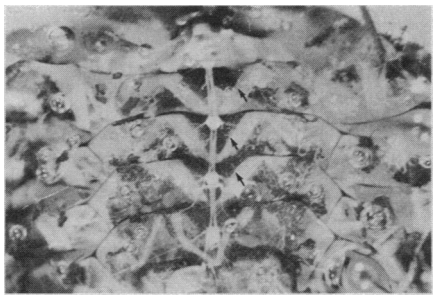
There is behavioral evidence that organisms as diverse as bacteria (*1*, *2*), homing pigeons (*3–5*), and honey bees (*6*, *7*) are sensitive to earth strength magnetic fields. In magnetotactic bacteria the response to magnetic fields is based on intracytoplasmic magnetite (Fe_3O_4) particles that impart a permanent magnetic dipole moment to these prokaryotes (*1*, *2*). The sensory systems that detect magnetic fields in homing pigeons and honey bees are still unknown. However, reports of magnetite in both homing pigeons (*8*) and honey bees (*9*) as well as in other organisms (*10*, *11*) suggest that this iron oxide could also be the basis of magnetic field detection in eukaryotes. Since magnetite in honey bees is reported to be localized in the abdomen (*9*), we have histologically examined tissues of the honey bee abdomen and looked specifically for those cells that contain iron and for connections between these cells and the central nervous system, a requirement for a sensory receptor.

We have found bands of cells in each abdominal segment of the honey bee that contain numerous iron-rich granules. We have localized the cells and the granules by both light and electron microscopy. For light microscopy we stained with Prussian blue, a reaction in which iron forms a blue precipitate in the presence of acidic potassium ferrocyanide. For electron microscopy we relied on electron opaqueness coupled with x-ray microanalysis to identify the iron granules. Using Mössbauer spectroscopy we identified the iron granules as consisting principally of hydrous iron oxides.

For gross examination of the honey bee abdomen (Fig. 1), adult foraging workers were pinned on dental wax, and their abdomens were cut and pinned open with stainless steel minutien pins. The abdominal contents were fixed and stained in situ (*12*). The dissected abdomens were then washed in distilled water and examined with a Zeiss dissecting microscope. The stained cells (Fig. 1) occur in a band under the epidermis in each abdominal segment. There is a higher concentration of cells in the ventral abdomen under each segmental ganglion. Sheets of this tissue were removed; examination revealed that the iron-positive staining cells form a reticular network within which is another population of smaller spherical nonstaining cells. In fresh, unstained tissue these two cell types are easily distinguished, and the iron-containing cells are seen to have granules of a yellowish color.

Examination of dissected whole abdomens stained with methylene blue shows that at each segmental ganglion a small nerve branch enters into the iron-positive tissue layer and ramifies throughout it. Mechanical movement of this small nerve trunk causes movement of this tissue layer but no other observable structures. Thus, it seems that this tissue is supplied with an efferent or afferent nerve supply (or both).

For light microscopy tissue was fixed and embedded in plastic. Sections (3 μm thick) were mounted on slides and stained for iron with the acidic potassium ferricyanide. The sectioned material (Fig. 2) shows the two cell types that



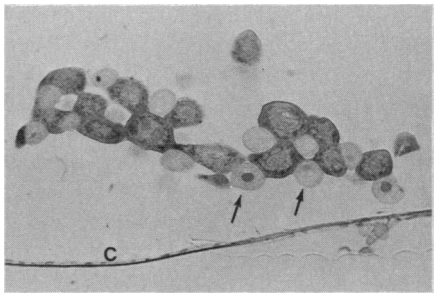


Fig. 1 (left). Honey bee abdomen pinned open and stained for iron. Arrows show large groups of iron-staining (dark) cells under segmental ganglion. Fig. 2 (right). Light photomicrograph of a section of the ventral portion of an abdominal segment containing oenocytes and fat cells (arrows). The oenocytes are characterized by a granular staining pattern; *c*, cuticle.

0036-8075/82/1112-0695$01.00/0

Cotransmitters in the motor nerves of the guinea pig vas deferens: electrophysiological evidence

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Science **218** (4573), 693-695.
DOI: 10.1126/science.6291151