was relatively long. The form of the clavus with a straight line of separation is indicative of the suborder Hemiptera.

The venation of the preserved portion of the wing is very unusual (Fig. 2). The stem of the radius is slightly bowed and subparallel to the costal margin, and the stems of *CuA* and *M* are fused toward their bases for some distance. In most Homoptera the stem of *R* is either straight or else bowed away from the anterior margin, and the stems of *M* and *CuA* are usually not fused, although they may be, for example, in many Recent Membracidae.

The combination of a radius bowed toward the fore margin and the distinct basal fusion between the stems of *M* and *CuA* occurs in the Stenoviciidae, a family of unusual small Homoptera, well represented in the Upper Permian of Australia and recorded also from the Permian of Russia and the Triassic of Australia. The fossil is tentatively referred to this family.

This Antarctic homopteran wing resembles those of certain Newcastle Coal Measures insects and possibly also some from the Bowen Basin of Queensland, Australia. Both of these have conchostracan beds of Permian age. Thus, the postulation of proximity of Antarctica and Australia, suggested by fossil clam shrimp from the Ohio Range (*8*), gains another bit of support from this Sentinel Mountain find.

PAUL TASCH

Department of Geology, Wichita State University, Wichita, Kansas 67208

EDGAR F. RIEK

Division of Entomology, Commonwealth Scientific and Industrial Research Organization, Canberra, A.C.T., Australia

References and Notes

1. The insect-bearing slab was collected by P.T. from the Polarstar Formation, Sentinel Mountains, during 1966–67 austral season and is described by E.F.R.
2. P. Tasch, *Antarctic J. U.S.* **2(4)**, 112 (1967).
3. C. Craddock, T. W. Bastian, R. H. Rutford, J. J. Anderson, *Science* **148**, 634 (1965).
4. P. Tasch, *Trans. Kansas Acad. Sci.* **71(2)**, 184 (1968).
5. E. P. Plumstead, in *Antarctic Geology, Proc. Int. Symp. Antarctic Geol. 1st*, R. J. Adie, Ed. (Interscience, New York, 1965), pp. 637–654; p. 644, column 2.
6. P. Tasch, presented during AAAS Symposium on Antarctic Research, Dallas, Texas, 1968.
7. Repository: U.S. Nat. Mus., No. 162006, Catalog 33.
8. P. Tasch, *Trans. Kansas Acad. Sci.* **70(2)**, 151 (1967).
9. J. L. Miller, (Wichita State University), H. H. Ross (Illinois Natural History Survey), and E. MacLeod (University of Illinois) were consulted upon discovery of the wing in slab S-10. Supported by NSF grant GA-519 to P.T. Logistic air support provided by U.S. Navy.

10 March 1969; revised 21 April 1969

Generation of Adrenergic and Cholinergic Potentials in Sympathetic Ganglion Cells

Abstract. *Norepinephrine elicited a hyperpolarizing response, and acetylcholine (during nicotinic blockade) elicited a depolarizing one. Both responses showed no increase in membrane conductance. The norepinephrine response was suppressed by initial depolarization; the acetylcholine response (frog cells); by hyperpolarization. These neurotransmitters apparently can activate electrogenic mechanisms which do not involve movement of ions down their electrochemical gradients.*

Sympathetic ganglion cells can respond to suitable input of preganglionic impulses not only with the well-known fast excitatory postsynaptic potential (EPSP) but also with two much slower postsynaptic potentials (*1*), one hyperpolarizing or inhibitory (slow IPSP), the other depolarizing (slow EPSP) (Fig. 1A). The membrane mechanisms involved in generating the slow PSP's are different in principle (*2*) from those for the fast EPSP's and IPSP's (*3*). We now report that norepinephrine (NE) can elicit in these cells a hyperpolarizing response that has unique characteristics similar to those already found for the slow IPSP (*2*); that is, the response is not accompanied by any increase in membrane conductance and it is suppressed [rather than increased (*3*)] by an initial depolarization of the resting membrane potential. In addition, acetylcholine (ACh), in the presence of strong nicotinic blocking agents, still elicits a depolarizing response which has the unique characteristics of the slow EPSP (*2*); that is, the response is accompanied by either little or no change (rabbit cells) or a decrease (frog cells) in membrane conductance, and it is suppressed or reversed in polarity (frog cells) by an initial hyperpolarization [rather than by a depolarization (*3*)] of the resting membrane potential. The choice of these neurotransmitter substances followed from the proposals that the slow IPSP is directly mediated by an adrenergic transmitter (*1, 4*) and that the slow EPSP is mediated by ACh acting at muscarinic postsynaptic receptor sites (*4, 5*). There are already reports which indicate that adrenergic agents can produce the postulated hyperpolarizing effect (*1, 6*) and that ACh can produce the postulated muscarinic depolarizing action (*7, 8*); we have studied this point further in the single cell.

The methods used for intracellular recording in amphibian and mammalian ganglion cells (*1*) and for passing currents via the recording microelectrode either for measuring changes in ohmic resistance or for steady polarization of the membrane (*2*) have been described (*9*). The superior cervical ganglia of young rabbits were studied at 35° to 37°C; the most caudal ganglia (9th or 10th) of the bullfrog's paravertebral sympathetic chain were studied at room temperature (about 20° to 22°C). We elicited responses to the neurotransmitters by adding a small volume (0.3 ml) of the test solution to the 30-ml chamber, which already contained a monoamine oxidase inhibitor (harmine, 5 μg/ml) for NE tests or an anticholinesterase (eserine sulfate, 2 μg/ml) for ACh tests. To obtain, in frog ganglia, responses to ACh which have relatively little or no contamination by the nicotinic action of ACh, the blocking agent nicotine ($10^{-4}M$) was added at least 30 minutes before the ACh tests (*10*); in rabbit ganglia, *d*-tubocurarine (50 μg/ml) was added earlier for the same purpose (*5*).

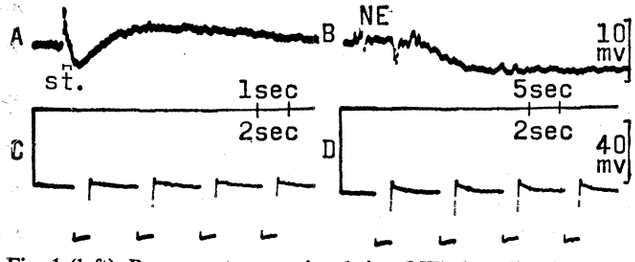
Addition of NE ($1.5 \times 10^{-4}M$) elicited a hyperpolarizing response (Fig. 1B) in 11 out of 12 resting ganglion cells tested under the above conditions in rabbit ganglia (*11*). The response averaged about 4 mv of hyperpolarization (from resting potentials of about 50 mv); this value is not far from the average peak amplitude for the slow IPSP itself (*1*). The hyperpolarization induced by NE was sustained at a steady level, at least for the duration of observation lasting some minutes. Ohmic resistance of the membrane measured during the NE response was not significantly different from that measured before addition of this compound (Fig. 1, C and D). (Ohmic resistance of the membrane was indicated by the height of the voltage change across the membrane produced by long-lasting pulses, usually 1-second, of constant current.) An initial steady depolarization of the membrane had the unique effect of depressing the hyperpolarizing response to NE, as found for the slow IPSP (*2, 12*). In nine cells, in which a steady depolarization of 20 mv from the resting level depressed the slow

IPSP itself to a negligible remnant of the control value, the mean response to NE was only about 0.4 mv when tested during such depolarization; this should be compared to the mean value of 4 mv for the response to NE in resting cells. The standard error of the difference of the mean responses for the two groups of cells, resting and depolarized, is 0.61. The *t*-test for significance of the difference of the means indicates a probability for such a difference that is considerably less than .001.

Addition of ACh ($10^{-3}M$), with only eserine present, caused a large sustained depolarization of about 35 mv in rabbit ganglion cells and of about 15 mv in frog cells (Fig. 2B). This was in part or predominantly a nicotinic action of ACh as it was not blocked by atropine. These depolarizing responses in the cells of both species were accompanied by a marked decrease in membrane resistance (Fig. 2, A and B). Evidence for such an expected increase in conductance (*3*) has already been obtained for nicotinic depolarization in frog ganglion cells (*8*), and for the initial EPSP (*2, 13*).

Addition of ACh ($10^{-3}M$), with a nicotinic blocker as well as eserine present, produced about 10- to 15-mv depolarization in mammalian and amphibian ganglion cells (Fig. 2, C–E). There was no decrease in membrane resistance, however, during this muscarinic depolarizing response. On the contrary, a marked increase in resistance of up to more than twice the resting value occurred in the frog cells (Fig. 2, C–E); in rabbit cells there was only a very slight increase, if any, in membrane resistance. During the course of the slow EPSP, elicited by preganglionic stimulation, increase in membrane resistance of 50 to 100 percent could also be observed in frog ganglion cells treated with nicotine and eserine (Fig. 2F). With eserine absent but nicotinic blockade present, both the depolarizing response and the accompanying increase in membrane resistance were smaller, whether produced by ACh or preganglionic stimulation (see also *2*). An initial steady 10- to 30-mv hyperpolarization from the resting membrane potential completely suppressed the muscarinic depolarizing response to ACh (nicotine and eserine present). In fact, ACh produced a temporary hyperpolarizing response in most of the nicotinized cells during such a steady hyperpolarization; a similar reversal of polarity in the slow EPSP was observed in these same cells when the slow EPSP was elicited during steady hyperpolarization of the membrane.

The argument may be raised that inability to detect an increase in membrane conductance during response to NE or ACh (muscarinic) may be due to location of the appropriate receptor sites at points remote from the recording-testing microelectrode (see *14*), in contrast to the location of receptor sites for the nicotinic depolarizing response to ACh. This argument has already been considered with respect to the similar difference between the slow PSP's and the fast EPSP in these cells (*2*); the same considerations apply to the NE and ACh actions and need not be repeated here in full. Frog ganglion cells are unipolar and lack the dendritic branching needed to provide remote receptor sites (see *2*); in addition, the muscarinic ACh action elicits a considerable conductance change in these cells, but it is opposite in direction to that of the nicotinic action. In rabbit cells, the same amplitudes of steady polarizing currents applied through the recording electrode for altering the fast EPSP were also sufficient for altering the response to NE (as well as for affecting the slow IPSP and slow EPSP); the same was true for all the responses in frog cells (*15*). All this indicates that, when present at all, the receptor sites for nicotinic ACh, muscarinic ACh, and NE actions are not far apart electrically. Therefore, absence of a detectable increase



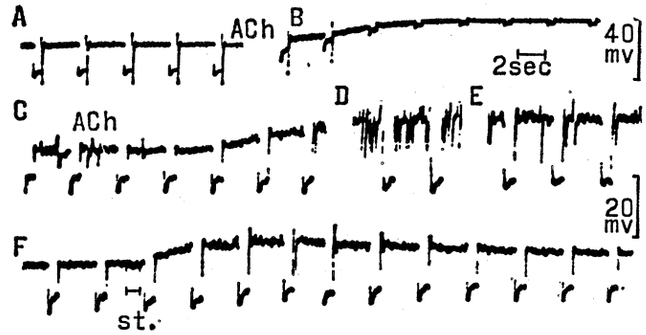


Fig. 1 (left). Response to norepinephrine (NE), in cells of rabbit's superior cervical ganglion, already treated with *d*-tubocurarine (15 μg/ml) and with harmine present (5 μg/ml). (A) Response to brief train of stimuli, 40 per second (bar *st*) to preganglionic nerve, showing the summated fast EPSP's during stimulation, with superimposed slow IPSP developing during and after train, followed by the longer lasting slow EPSP. (B) Same cell as in A, in resting state, NE (as bitartrate) added to chamber (50 μg/ml) as indicated by the mechanical artifacts; prolonged hyperpolarization develops several seconds later. (C) Another cell, in resting state, with hyperpolarizing pulses of constant current to measure membrane resistance; the difference between the top base line, at external potential, and that of the intracellular electrode indicates the resting membrane potential, of about 50 mv. (D) Same as in C, but taken 4 minutes after addition of NE which produced a steady hyperpolarizing response of about 6 mv. Time calibration shown for each recording; voltage calibration in B for A and B, in D for C and D.

Fig. 2 (right). Responses to acetylcholine (ACh), in B cells of frog's sympathetic ganglion; eserine (2 μg/ml) present. (A) Resting cell with pulses for testing membrane resistance. (B) Same as A, but record begins about 25 seconds after addition of ACh ($10^{-3}M$) to bath; note the developing depolarization, up to about 15 mv at the steady level, and the decreasing amplitude of the testing pulses, indicating a lowered resistance. (C) Another cell, with nicotine ($10^{-4}M$) as well as eserine present; ACh ($10^{-3}M$) added after the first two resistance-testing pulses. (D) Continuation of record a few seconds after C. (E) Continuation of D after an interval of about 60 seconds. Resistance increases during this muscarinic depolarization; some discharge of action potentials (tops of spikes off screen) can be seen in D and E. (F) Same cell before C and E, responding to a 1-second train of preganglionic stimuli at 80 per second (delivered during *st*) to show its slow EPSP response; the absence of summated fast EPSP's during the stimulus is due to full nicotine blockade, whereas the absence of any slow IPSP is a demonstration of the finding (*1*) that the B neurons in frog ganglia do not exhibit slow IPSP, in contrast to the C neurons. The time calibration was the same for all; the voltage calibration is shown in B for A and B, and in F for C through F.

in conductance during the muscarinic ACh and the NE actions reflects a real difference in membrane response from that during the nicotinic ACh actions (*16, 17*). In addition, the effect of initial depolarization on the NE response (rabbit cells) and of initial hyperpolarization on the muscarinic ACh response (frog cells) are opposite in direction to those expected from the ionic hypothesis for electrogenesis of such responses (see *3*). We conclude, therefore, that neither the hyperpolarizing action of NE nor the muscarinic depolarizing action of ACh on sympathetic ganglion cells is mediated by the generation of membrane currents that are due to ions moving down their electrochemical gradients.

Activation of an electrogenic Na-K pump could provide one alternative possibility for generating the hyperpolarizing response to NE without an increase in membrane conductance (*2, 12, 17*). However, we found that ouabain ($10^{-5}M$), a specific inhibitor of such an active transport mechanism for Na coupled to K intake (*18*), did not block the response to NE at a time when ouabain had already depressed the sodium pump; depression of the sodium pump was shown by changes in the cell's action potential due to accumulation of Na^+ (*19*). This evidence argues against at least the ouabain-sensitive type of sodium pump as the electrogenic source of either the NE response or, by implication, the slow IPSP in these cells (*17*).

The electrogenic mechanisms for both the NE and the muscarinic ACh responses are still to be worked out. However, the findings of the unique similarities of the NE and muscarinic ACh mechanisms to those mediating the slow IPSP and slow EPSP, respectively, provide strong additional support for the proposals that NE or a closely related substance is the synaptic mediator for the slow IPSP (*1, 4*), and that ACh is the mediator for the slow EPSP in these cells (*4, 5*). Furthermore, NE and ACh, both compounds of physiological and pharmacological importance, can elicit postsynaptic responses by physicochemical mechanisms different in principle from those which mediate the better-known synaptic transmitter actions (*3*).

BENJAMIN LIBET
HARUO KOBAYASHI*
Department of Physiology, University of California School of Medicine, San Francisco 94122

References and Notes

1. B. Libet and T. Tosaka, *Fed. Proc.* **25**, 270 (1966); T. Tosaka, S. Chichibu, B. Libet, *J. Neurophysiol.* **31**, 396 (1968); B. Libet and T. Tosaka, *ibid.* **32**, 43 (1969).
2. H. Kobayashi and B. Libet, *Proc. Nat. Acad. Sci. U.S.* **60**, 1304 (1968); B. Libet and H. Kobayashi, *Fed. Proc.* **27**, 750 (1968).
3. J. C. Eccles, *The Physiology of Synapses* (Springer, Berlin, 1964), pp. 49–53 and 173–188.
4. R. M. Eccles and B. Libet, *J. Physiol. (London)* **157**, 484 (1961).
5. B. Libet, *J. Neurophysiol.* **30**, 494 (1967); ———, in *Studies in Physiology*, D. Curtis and A. K. McIntyre, Eds. (Springer, Heidelberg, 1965), p. 160.
6. W. C. De Groat and R. L. Volle, *J. Pharmacol.* **154**, 1 (1966).
7. C. Takeshige and R. L. Volle, *Brit. J. Pharmacol.* **23**, 80 (1964).
8. K. Koketsu, S. Nishi, H. Soeda, *Life Sci.* **7**, 741 (1968).
9. H. Kobayashi and B. Libet, in preparation.
10. B. L. Ginsborg and S. Guerrero, *J. Physiol. (London)* **172**, 189 (1964).
11. Only a few cells tested in frog ganglia responded to NE or to epinephrine, but these were all B neurons (*1*), which receive preganglionic B fiber input. The B neurons do not exhibit any slow IPSP (*1*) and would therefore not be expected to respond necessarily to NE or to epinephrine. The C neurons consistently exhibit slow IPSP (*1*), but a successful impalement of these cells is a rarity. The study of the characteristics of the NE responses was therefore confined to the rabbit ganglia, in which most cells do exhibit slow IPSP (*1*).
12. S. Nishi and K. Koketsu, *J. Neurophysiol.* **31**, 717 (1968).
13. ———, *J. Cell. Comp. Physiol.* **55**, 15 (1960); J. G. Blackman, B. L. Ginsborg, C. Ray, *J. Physiol. (London)* **167**, 355 (1963).
14. T. G. Smith, R. B. Wuerkar, K. Frank, *J. Neurophysiol.* **30**, 1072 (1967).
15. P. G. Nelson and K. Frank, *ibid.*, p. 1097.
16. K. Krnjević and S. Schwartz [*Exp. Brain Res.* **3**, 306 (1967)] have reported that the atropine-sensitive depolarizing response of cerebral pyramidal cells to ACh can occur without a detectable increase in membrane conductance. No further evidence was available to help eliminate the possibility that this result was explainable by a remote location of the ACh receptor sites. But their finding is at least consistent with the possible existence in brain of an electrogenic mechanism similar to that for the slow EPSP in sympathetic ganglia (*5*).
17. H. Pinsker and E. R. Kandel [*Science* **163**, 931 (1969)] have recently reported that neurons in *Aplysia* ganglia can exhibit a cholinergically mediated slow IPSP with no change in membrane conductance; however, this response, whether elicited by neural action or applied ACh, was selectively blocked by ouabain.
18. J. C. Skou, *Physiol. Rev.* **45**, 596 (1965).
19. In addition, G. L. Gebber and R. L. Volle [*J. Pharmacol.* **152**, 18 (1966)] have reported that the hyperpolarizing response to epinephrine was not blocked by ouabain when the latter had depressed the positive afterpotential that follows spike discharge.
20. Supported in part by NIH grant NB-00884.
* Present address: Department of Physiology, Tokyo Medical College, Shinjuku-ku, Tokyo.

2 February 1969

Immunoglobulins G, A, and M Determined in Single Cells from Human Tonsil

Abstract. *The analysis of specific proteins in single cells is described and applied to the determination of immunoglobulins G, A, and M (γG, γA, and γM) in lymphoid cells of the human tonsil. The number of cells containing γA or γM was much less than the number of cells containing γG, but the average cell content of γA or γM was much greater than the average cell content of γG. Similarly, in one child where relatively few cells containing γG were found, the average cell content of γG in those cells was higher than that in the other children. The limit of sensitivity of the method for each of the three immunoglobulins with the antiserums used was less than 10^{-14} gram of the specific protein.*

A general method for the determination of specific proteins in single cells forms the basis for the estimation of immunoglobulins G, A, and M (γG, γA, and γM) in lymphoid cells obtained from tonsillar tissue at tonsillectomy of otherwise normal children aged 2½ to 8 years. Cells were teased from the fresh tissue and washed four times in Alsever's solution (cells washed from two to five times gave the same results); virtually all of the nucleated cells in these preparations were lymphoid. A given number of cells in 20 μl of Alsever's solution, or approximately 5×10^5 lymphoid cells, was spread over the surface of a mixture of antiserum and agar which had been poured to a depth of approximately 2 mm between two glass strips placed 25 mm apart on a microscope slide (2.5 × 7.5 cm) (*1*); the glass retainers were 10 by 25 mm strips cut from other microscope slides, and the agar surface formed between them was a square about 25 mm on a side. The antiserum-agar mixture contained, per 100 ml of mixture, 1.5 g of agar, 0.1*M* sodium borate buffer of *p*H 8.6, 0.8 g of sodium deoxycholate, and a given amount of rabbit antiserum specific for either γG, γA, or γM. The deoxycholate was essential for eventual lysis of all cells coming to rest on the agar as the suspending solution evaporated and for the subsequent release of cellular immunoglobulin (*2*); simple lysis of cells by freezing and thawing or by sonic vibration did not free immunoglobulin from the cells. The slides were kept in a humid chamber for 6 days, after which the agar was covered with a square of cellulose acetate mem-