ibid., pp. 175–193]. Of these, PTP is the most persistent. In the hippocampal slice, PTP lasts 1 to 4 minutes in response to the repetitive stimulation parameters that we use. Long-term potentiation (LTP) is operationally defined as an enhancement that clearly outlasts PTP (see text) and that can be induced by repetitive synaptic stimulation for seconds or less [W. B. Levy and O. Steward, *Neuroscience* **8**, 797 (1983); G. Barrionuevo and T. H. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7347 (1983); C. Briggs, T. H. Brown, D. A. McAfee, *J. Physiol. (London)* **359**, 503 (1985); D. A. Baxter, G. D. Bittner, T. H. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5978 (1985)].

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6. Experimental procedures are described fully in (*4*). The electrode arrangement shown in Fig. 1A appeared to result in activation of separate sets of afferent fibers by the different electrodes. This was based on (i) paired-pulse facilitation tests, which were performed with the same or a different electrode for the second (test) pulse [(*4*) and also assessed in the present experiments], (ii) an absence of heterosynaptic LTP [(*4*) and present results] and (iii) the demonstrated specificity of the associative synaptic enhancement (present results). Subthreshold population excitatory postsynaptic potential waveforms were digitized (5 kHz sampling rate) for on-line computer analysis (IBM PC-XT) of peak amplitudes.
7. Heterosynaptic LTP does not occur in region CA1 [G. Lynch, T. Dunwiddie, V. Gribkoff, *Nature (London)* **266**, 737 (1977); P. Andersen *et al.*, *ibid.*, p. 736; [(*4*) and present results].
8. The two stimulation patterns differ only (Fig. 1B) in the identity of the W electrode selected for forward pairing with the S electrode (the one closest to area CA3 or the one closest to the S electrode). In these experiments, the S electrode was always located toward the subiculum (Fig. 1A). We obtained similar results when the S electrode was located toward the CA3 region.
9. Generally only one of the two stimulation patterns was applied to each slice. For illustration, Fig. 1C shows both patterns used in succession. We have not applied the two patterns in succession a sufficient number of times to comment on the effect of sequential applications.
10. Stimulation of W alone produces PTP but not LTP (*4*). Unpaired S stimulation either produces a brief heterosynaptic depression or has no effect on the W responses (*4*). The amplitude of the S response was only monitored immediately before each set of trains when stimulation parameters were readjusted to elicit a 2.5-mV S response.
11. All tests were paired t tests for dependent means. They were one-tailed because the a priori hypothesis and past experience lead to a unidirectional prediction—a post-pairing enhancement or no change.
12. The temporal contiguity requirements for associative LTP are considered elsewhere [S. Kelso and T. H. Brown, *Soc. Neurosci. Abstr.* **10**, 78 (1984); S. Kelso and T. H. Brown, in preparation]. Explicitly temporal aspects of the role of the hippocampus in conditioning have been proposed to engage and require a variety of neural feedback loops, possibly including some form of efference copy [E. Halgren, in *Neuropsychology of Memory*, L. R. Squire and N. Butters, Eds. (Guilford, New York, 1984), pp. 165–182; R. F. Thompson *et al.*, *ibid.*, pp. 424–442; T. Berger, *ibid.*, pp. 443–461; J. W. Moore and P. R. Solomon, *ibid.*, pp. 462–488].
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14. The conjunctive mechanism responsible for the differential induction of associative LTP has been examined by substituting for the usual S input intracellular current- and voltage-clamp procedures that forced or prevented a correlation between pre- and postsynaptic activity (S. R. Kelso, A. H. Ganong, T. H. Brown, *Proc. Natl. Acad. Sci. U.S.A.*, in press). The results suggest that the role of the S input is simply to provide a critical amount of postsynaptic depolarization in the proper temporal relationship to activity in the W inputs.
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Vasoconstriction: A New Activity for Platelet-Derived Growth Factor

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Platelet-derived growth factor (PDGF) is a potent mitogen for vascular smooth muscle cells that has been implicated in the pathogenesis of atherosclerosis. The potential role of PDGF in the altered vasoreactivity of atherosclerotic vessels has been studied through an examination of its effects on contractility in the rat aorta. PDGF caused a concentration-dependent contraction of aortic strips and was significantly more potent on a molar basis than the classic vasoconstrictor peptide angiotensin II. Furthermore, PDGF increased the cytosolic free calcium concentration in cultured rat aortic smooth muscle cells. These observations suggest a new biological activity for PDGF that may contribute to the enhanced vasoreactivity of certain atherosclerotic vessels.

PLATELET-DERIVED GROWTH FACTOR (PDGF), a cationic protein of platelet alpha granules, is a potent mitogen in vitro for vascular smooth muscle cells (VSMC) (*1*). In addition, PDGF in vitro causes migration of VSMC as well as of fibroblasts and inflammatory cells (*2*). On the basis of these activities it has been proposed that PDGF, released at sites of vascular injury, plays a significant role in the development of atherosclerosis, particularly in VSMC migration into and proliferation in the intima (*3*). Although contraction and maintenance of tone are the major physiologic functions of VSMC, the consequences of mitogenic stimulation, as occurs in atherosclerosis, on these physiologic functions of VSMC have not been considered. Because both experimental and clinical coronary artery spasm occur predominantly at the site of atherosclerotic lesions (*4*) and because atherosclerotic lesions are thought to have increased basal tone (*5*), it has been suggested that some features of this disease are responsible for the hypercontractility seen basally and in response to agonists such as ergonovine, histamine, and serotonin (*6*). We have proposed that mitogenic influences acting on VSMC in atherosclerotic arteries may contribute to the enhanced contractile responsiveness (*7*). This seems plausible because (i) certain mitogens (including PDGF) mobilize intracellular calcium in several cell types (*8*), and (ii) agonist-mediated increases in cytoplasmic calcium result in a contractile response in VSMC (*9*). PDGF is the mitogen generally considered to be the most important in stimulating the proliferation of VSMC in atherosclerosis (*3*). To test the hypothesis that PDGF can mediate contraction in VSMC, we assessed its contractile effects on rat aorta and its ability to mobilize calcium in cultured rat VSMC. To evaluate the relative potency of PDGF as an agonist, we compared its vasoactive effects with those of the potent vasoconstrictor angiotensin II.

The effects of purified human PDGF on contractile tension in isolated rat aortic strips are illustrated in Fig. 1. The PDGF used in these experiments was purified to more than 99 percent homogeneity accord-

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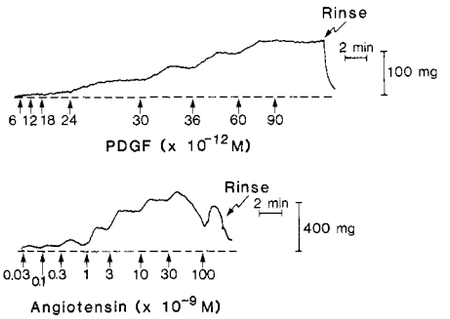

Fig. 1. Contraction of isolated rat aorta induced by PDGF or angiotensin II. Helical strips of rat aorta were prepared and allowed to equilibrate for 2 hours in a muscle bath (*7*). All strips had intact endothelium as assessed by vasodilation on exposure to acetylcholine. The strips were placed at an optimal length for the development of active force in response to norepinephrine. Contractile responses to PDGF or angiotensin II were recorded after sequential addition of increasing amounts of either agonist to the bath. Concentrations are expressed as the cumulative molar concentration. Tracings are representative of ten separate experiments.

ing to the technique of Raines and Ross (*10*). Cumulative addition of PDGF to the muscle bath produced concentration-related contractile responses in all strips tested. At the highest concentration of PDGF tested (0.09 n*M*), approximately 135 mg of tension was generated, which was about 30 percent of that caused by angiotensin II (100 n*M*) (Fig. 1). PDGF-induced contraction was readily reversible after incubation for periods as long as 30 minutes. Endothelium was shown to be intact in the aortic strips before every experiment by the induction of relaxation in response to acetylcholine. For some experiments, endothelium was intentionally rubbed off, but no change in response to a maximal dose of PDGF was observed. This contrasts with the augmented vasoconstriction seen in the presence of denuded endothelium in response to such agonists as norepinephrine (*11*). The vasoconstrictor response to PDGF was not affected by the following antagonists: ketanserine ($3 \times 10^{-7} M$), phentolamine ($10^{-6} M$), or indomethacin ($2 \times 10^{-6} M$). PDGF had no effect on acetylcholine-induced relaxation of aortic strips contracted by a dose of serotonin having 30 percent of the maximal effect (EC_{30}), suggesting that PDGF had no effects mediated by endothelium in this preparation (*12*). Removal of extracellular Ca^{2+} abolished the PDGF-induced contractile response. Experiments to investigate the existence of a possible synergistic effect of PDGF on vasoconstriction mediated by angiotensin II were carried out, but, because rat aorta developed tachyphylaxis in response to repeated administration of angiotensin II, such an effect was not demonstrable. However, PDGF increased contractions of rat aortic strip induced by EC_{30} doses of either serotonin ($4.5 \times 10^{-8} M$) or $PGF_{2\alpha}$ ($4.9 \times 10^{-7} M$) in an additive, but not potentiated, fashion (*13*).

Concentration-response relationships for PDGF- and angiotensin II–induced contraction are depicted in Fig. 2. The concentration of angiotensin II having a half-maximal effect (EC_{50}) was approximately 5.9 ± 1.3 n*M*, whereas the EC_{50} for PDGF was about 0.028 ± 0.002 n*M*. Maximal PDGF-induced contraction (130 mg) was approximately 30 percent of that produced by the classic vasoconstrictor peptide angiotensin II. On a molar basis, PDGF is a potent vasoconstrictor, inducing 100 mg of tensile force at 0.036 n*M*, whereas angiotensin II achieved similar tension at 1.6 n*M*, a suprapysiologic concentration (*14*).

The concentrations of PDGF used for the experiments reported here are in the same range as those thought to be present in serum (*1*). Although probably higher than the levels of PDGF in circulating blood, one might anticipate that concentrations similar to those used here might be attained locally at sites of active platelet-vessel interactions, as has been shown for platelet factor 4 (*15*). PDGF has been reported to be fully mitogenic for VSMC and other cell types at concentrations between 0.03 and 0.20 n*M* depending on culture conditions (*1, 10*), concentrations that significantly contract rat aorta (Fig. 2). The overlap between the concentrations of PDGF that induce growth and contraction supports the hypothesis that mitogenic stimulation of VSMC in active lesions of atherosclerotic arteries may result in increased contractile activity. It also seems probable that these two activities of PDGF result from certain shared intracellular mechanisms of action. Mobilization of calcium in VSMC is likely to be such a common cellular mechanism.

To study the effects of PDGF and angiotensin II on VSMC calcium homeostasis, we used the fluorescent probe quin2 to monitor changes in cytosolic free calcium concentration ($[Ca^{2+}]_i$) in cultured rat aortic VSMC (Fig. 3). PDGF (0.13 n*M*) caused a transient increase in $[Ca^{2+}]_i$ after a brief initial lag period. The $[Ca^{2+}]_i$ reached a maximum value (about a threefold increase) at 40 seconds and returned close to prestimulus levels within 8 minutes. In contrast, 10 n*M* angiotensin II, as previously reported (*16*), caused a rapid increase in $[Ca^{2+}]_i$, which reached peak values of five to six times the resting level within 15 seconds and then showed a gradual decline to a new plateau at two to three times the prestimulus level. PDGF increased $[Ca^{2+}]_i$ in a concentration-dependent manner with threshold effect at 0.03 n*M*. Maximal PDGF or angiotensin II concentrations in several preparations induced five- to tenfold increases in $[Ca^{2+}]_i$. However, since quin2 fluorescence was nearly saturated at these high concentrations of agonist, the calculated increase in $[Ca^{2+}]_i$ may be underestimated. The concentrations of PDGF that increase $[Ca^{2+}]_i$ in cultured VSMC were similar to those that induced aortic contraction (Fig. 2) but are considerably lower than those reported to stimulate intracellular Ca^{2+} release in fibroblasts (*17*). The signal transduction mechanisms for PDGF receptor binding and Ca^{2+} mobilization in cultured rat aortic VSMC may be different from PDGF-induced contraction of rat aortic strips. Thus, cultured VSMC are noncontractile cells under the conditions used for these studies. In addition, according to data presented by Chamley-Campbell and Campbell (*18*), freshly dissociated VSMC from rat aorta would not be expected to show a mitogenic response to PDGF. These workers found that aortic VSMC in the first few days of primary culture are in a contractile phenotype and do not divide when 5 percent serum is added. After 6 to 8 days the cells change their phenotype such that they no longer contract but respond to the mitogens present in serum with logarithmic growth. Possible explanations for these data are a lack of PDGF receptors in VSMC when initially isolated or an inability of VSMC to give a mitogenic response to PDGF while in the contractile phenotype. In view of these data, the response of aortic strips to PDGF demonstrated here represents evidence, albeit indirect, for the existence of PDGF receptors in VSMC in vivo.

To examine further the mechanisms by which PDGF mobilizes Ca^{2+} in VSMC, we

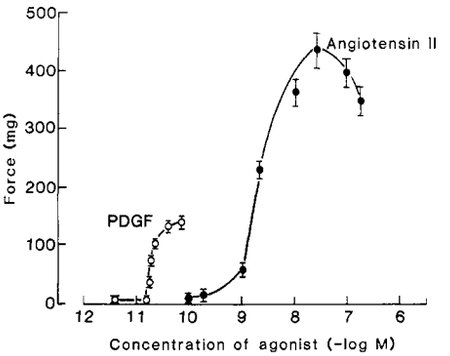

Fig. 2. Dose-response curves for PDGF- and angiotensin–induced rat aortic contraction. Each experimental value is the mean ± SEM of six determinations. EC_{50} values were estimated by logit-log transformation after normalization to the percentage of maximal response for a total of ten strips.

repeated the experiments in the presence of EGTA to chelate external Ca^{2+}. Exposure to 2 m*M* EGTA for 5 minutes produced a small decrease in resting $[Ca^{2+}]_i$ (Fig. 3C) relative to basal levels (Fig. 3A), after which addition of PDGF caused a transient 50 percent increase in $[Ca^{2+}]_i$, suggesting that Ca^{2+} is released from an intracellular pool. However, the marked diminution of both the maximal increase in $[Ca^{2+}]_i$ and the plateau phase seen in the presence of external Ca^{2+} (compare Fig. 3A with Fig. 3C) suggests that the PDGF-mediated increase in $[Ca^{2+}]_i$ also depends on increased membrane permeability to extracellular Ca^{2+}.

The observation that a particular agonist induces a prolonged response despite a transient increase in $[Ca^{2+}]_i$, such as we found for PDGF, has been noted for other agonists in various cell types (*19*). To explain these findings, Rasmussen and co-workers have proposed, on the basis of their observations of angiotensin II–induced aldosterone secretion in adrenal glomerulosa cells, that angiotensin II stimulation of phosphatidylinositol 4,5-bisphosphate breakdown leads to the generation of two second messengers: inositol 1,4,5-trisphosphate (IP_3), which releases Ca^{2+} from an intracellular nonmitochondrial store, causing the rapid angiotensin II effect; and diacylglycerol (DG), which activates protein kinase C, contributing to the sustained phase of the angiotensin II response (*20*). This model may be relevant to the mechanism of PDGF-induced contraction of rat aorta since PDGF stimulates polyphosphoinositide breakdown and generation of IP_3 and DG in fibroblasts (*21*). However, although both PDGF and angiotensin II increase $[Ca^{2+}]_i$, we observed a slower onset and development of the PDGF-mediated effects (see Figs. 1 and 3, A and B). Furthermore, the PDGF response was inhibited to a greater extent by chelation of external Ca^{2+} than the angiotensin II response [compare figures 3C and 4 in (*7*)]. The differences in the temporal course of contraction, mobilization of $[Ca^{2+}]_i$, and dependence on extracellular Ca^{2+} for PDGF and angiotensin II may point to different patterns of activation of shared cellular mechanisms such as phopholipase C–mediated breakdown of phosphoinositides.

We have found that an anionic growth factor, epidermal growth factor (EGF), also induced rat aortic contraction with an EC_{50} of 19 n*M* (*7*). Our findings with EGF have been extended to rat ileocolic and superior mesenteric arteries by Muramatsu *et al.* (*22*) who found an apparent EC_{50} for contraction of 1.7 n*M*. They also reported that indomethacin (3×10^{-6} *M*) abolished this effect of EGF. The observations for EGF-mediated contraction contrast with those reported here for PDGF both in the much lower EC_{50} for PDGF (0.028 n*M*) and in the absence of an effect of indomethacin on contraction. Furthermore, EGF does not cause an increase in $[Ca^{2+}]_i$ that is detectable by quin2 in VSMC, a finding also reported for Swiss 3T3 cells (*23*). These observations thus suggest that, in addition to the striking differences in potency, PDGF and EGF differ in the mechanisms by which contraction is induced. Thus PDGF has features characteristic of conventional vasoconstrictor hormones that directly increase $[Ca^{2+}]_i$, whereas EGF may act through a prostaglandin intermediate without increasing $[Ca^{2+}]_i$ (*9, 22*). Two other partially purified growth factors, cationic fibroblast growth factor (FGF) and transforming growth factor β (TGF-β), were also assayed for contractile activity. Both growth factors stimulated contraction of rat aortic strips with a maximal contraction equivalent to 15 percent of that induced by angiotensin II. The EC_{50} doses for contraction were 2.86 ± 0.13 ng/ml and 3.52 ± 0.01 ng/ml for FGF and TGF-β, respectively (*24*).

We have demonstrated that PDGF is a potent vasoconstrictor for rat aorta and a calcium-mobilizing agonist for cultured rat aortic smooth muscle cells at concentrations that are strongly mitogenic for these cells. Our findings that PDGF, EGF, FGF, and TGF-β all stimulate contraction of rat aorta at concentrations in the range of nanograms per milliliter implies that vasoconstrictor activity may be a general feature of VSMC mitogens. The potency of PDGF in inducing contraction (EC_{50} of approximately two orders of magnitude lower than angiotensin II) is striking in view of the fact that angiotensin II has been considered to be the most potent vasopressor agent known, about 40 times as potent as norepinephrine (*25*). The similarities observed in the cellular effects of PDGF and angiotensin II suggest that certain mitogens and vasoactive agents may share intracellular mechanisms associated with increases in $[Ca^{2+}]_i$ and may therefore in a vascular smooth muscle cell result in contraction. The vasoconstrictor properties of PDGF represent a newly recognized biological activity for this mitogen. This activity, during periods of active mitogenic stimulation, may contribute to the abnormal vasoreactivity of atherosclerotic arteries (*5, 6*).

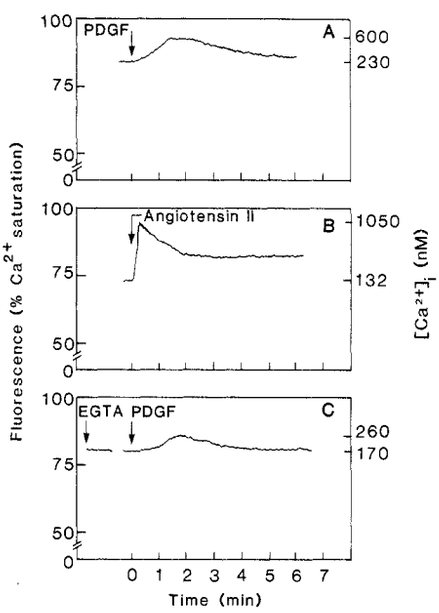

Fig. 3. Changes in $[Ca^{2+}]_i$ in cultured rat aortic VSMC induced by PDGF or angiotensin II. Quin2 loading and fluorescence monitoring of cultured rat aortic VSMC were done as described (*16*). The intracellular concentration of quin2 in rat aortic VSMC is about 1.5 m*M*, and the resting cytosolic free Ca^{2+} concentration is 198 ± 7 n*M* in buffer containing 1.5 m*M* Ca^{2+} (*16*). Tracings represent typical responses of quin2 fluorescence to addition (time zero) of (A) 0.13 n*M* PDGF or (B) 10.0 n*M* angiotensin II in the presence of 1.5 m*M* Ca^{2+}, and (C) 0.13 n*M* PDGF after chelation of extracellular Ca^{2+} by addition of EGTA (2 m*M*) for 5 minutes before PDGF was added.

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12. Helical strips of rat aorta were contracted by an EC_{30} dose of serotonin (4.5×10^{-8} *M*). Acetylcholine (5.5×10^{-7} *M*) was added and induced an

approximate 30 percent relaxation. The muscle bath medium was changed and serotonin ($4.5 \times 10^{-8}M$) and PDGF ($33 \times 10^{-12}M$) were added. After contraction reached maximal force, acetylcholine ($5.5 \times 10^{-7}M$) was again added and again induced a similar 30 percent decrease in tension.
13. Rat aortic strips were contracted by EC_{30} doses of either serotonin or $PGF_{2\alpha}$ which resulted in approximately 400-mg contractile tension. The muscle bath medium was changed, and after relaxation, an EC_{30} dose of the above agonists was added simultaneously with PDGF ($33 \times 10^{-12}M$). At maximal contraction, tension generation was approximately 460 mg of force, which represents summation of the effects of both agonists (Figs. 1 and 2).
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24. Partially purified TGF-β (prepared from human sources with estimated 80 percent purity) and cationic FGF (prepared from bovine pituitary with estimated 5 percent purity) were obtained from Biomedical Technologies Inc., Cambridge, MA. Helical strips of rat aorta were contracted as described in Fig. 1. Experimental values are mean ± SEM of four observations. EC_{50} values were estimated as described in Fig. 2. Based on a value of 5 percent for the purity of the cationic FGF fraction, the EC_{50} for FGF contraction would be 134 pg/ml, which is close to the EC_{50} value of 60 pg/ml reported for mitosis in VSMC in response to FGF [F. Esch *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6507 (1985)].
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Extended Oxygen Delivery from the Nerve Hemoglobin of *Tellina alternata* (Bivalvia)

DAVID W. KRAUS AND JAMES M. COLACINO

An oxygen-binding hemoglobin localized in the nerves of *Tellina alternata* (Bivalvia) required 30 minutes to unload oxygen when excised nerves were exposed to pure nitrogen. Neural excitability under these conditions could be sustained only until deoxygenation of the hemoglobin was complete. When the oxygen-combining function of the hemoglobin was abolished with carbon monoxide, the neural excitability ceased within a few minutes of oxygen removal, a response identical to that of hemoglobinless homologous nerves of other bivalves. These results demonstrate that aerobic activity can be supported by the oxygen stored on hemoglobin in microscopic tissues for a considerable time under anoxic conditions.

IF PIGMENTS CARRYING O_2 FUNCTION mainly to increase the availability of O_2 to respiring mitochondria, the molecular characteristics of the pigment should be matched to the magnitude and duration of O_2 delivery required by the tissue. As an example, myoglobin rapidly dissociates O_2 (*1*), exhibits high intracellular mobility (*2*), and has an O_2 affinity intermediate between those of hemoglobin and cytochrome oxidase (*3*). These properties enable myoglobin to facilitate O_2 diffusion during steady-state O_2 consumption (*4*) and to function as a short-term O_2 store (for a few seconds) at the onset of muscular contraction (*5*). Hemoglobins that slowly dissociate O_2 could not function in facilitated O_2 diffusion (*6*) or short-term storage. Instead, they could function as a long-term supply of O_2 if matched to a similar demand (*7*).

A few studies relating hemoglobin characteristics to animal behavior have indirectly demonstrated hemoglobin participation in extended O_2 supply (*8*). On the basis of O_2 unloading rates from the hemoglobin, body geometry, and O_2 consumption rates, we proposed that hemoglobin could be used for long-term O_2 storage in a microscopic animal, *Neodasys* (Gastrotricha) (*7*). By using continued nervous function as evidence for the presence of O_2 (*9*), we have found more direct evidence that the oxygen-carrying hemoglobin located in the ganglia and all major nerves of *Tellina alternata*, a deposit-feeding bivalve, is well suited to act as a long-term O_2 storage center. Under anoxic conditions the propagation of externally stimulated action potentials by the nerves continued until deoxygenation of the hemoglobin was complete (~30 minutes). If the hemoglobin was made nonfunctional with CO, the action potentials ceased shortly after ambient O_2 was removed (5 minutes), a response identical to that of hemoglobinless homologous nerves from a sympatric filter-feeding bivalve, *Tagelus plebeius*.

The nerve connecting the cerebral and visceral ganglia (the c-v nerve, which is typically 2 to 4 cm long, 0.012 to 0.016 cm in diameter, and surrounded by a tough, nonremovable collagenous sheath 0.0003 cm thick) was used for this study because it is relatively large and unbranched. The c-v nerve is densely packed with glial cells (red-pigmented in *T. alternata*) and slowly conducting axons (*10*), 0.1 to 0.3 μm in diameter (*11*). Hemoglobin characteristics in the excised nerve were determined on a microspectrophotometer with a specially constructed gas slide (*7*). A functional hemoglo-

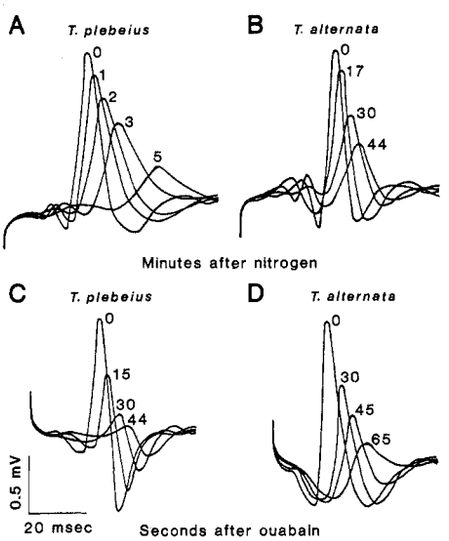

Fig. 1. Time course changes in action potential appearance under different conditions. Action potentials retraced from sequential photographs show a decrease in amplitude and conduction velocity until indistinguishable from baseline. A supramaximal stimulus of 4 to 7 V and 0.5 to 1.0 msec at 2 pulse/sec was used in (A) and (B) and 1 pulse/sec in (C) and (D); stimulus artifact is a vertical line at the left of the trace. The number at each peak indicates the time at which that trace was recorded after the treatment began.

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