signals have been recognized and discussed elsewhere (*5, 7, 9*). For the interpretations in this study, two of the most important of these factors are: (i) during the aequorin signal spatial gradients of calcium concentration [Ca^{2+}] may exist within the cell (*10*), and (ii) the relation between [Ca^{2+}] and aequorin luminescence is nonlinear (*11*). Thus, transient aequorin signals may not represent even an "average" cellular [Ca^{2+}], as would a linear Ca^{2+} indicator. For spatially uniform [Ca^{2+}] the limit of detection in these experiments has been estimated, by the method of Allen and Blinks (*7*), to be $10^{-7}M$ (*12*).

The best available evidence (*13*) indicates that at 35°C aequorin luminescence rises with a half-time ($T_{1/2}$) of 3 msec after a rapid increase in [Ca^{2+}]. Only the initial rise of the signal is rapid enough ($T_{1/2}$ = 9 msec) to have been even slightly affected by aequorin kinetics.

Potential problems arise in the present study because of the use of a multicellular, multiply injected preparation. If one assumes a conduction velocity of 2 m/sec, the temporal relation between membrane excitation and the aequorin signal (Fig. 1A) is probably not in error by more than ± 1.5 msec, since the electrically recorded cell and the other injected cells were never more than 3 mm apart. Similarly, the aequorin signal is probably not temporally dispersed by more than 3 msec.

The observed interval dependence of the initial rapid phase strongly suggests that an increase in Ca^{2+} during this part of the signal is related to the entry of Ca^{2+} through the surface membrane via the slow inward current (I_{si}). Voltage clamp experiments show that recovery of I_{si} in Purkinje fibers is a relatively rapid exponential process at resting membrane voltage (*3*) (τ = 0.67 second, 95 percent complete within 2 seconds; compare Fig. 2C). Although the interval dependence of peak I_{si} and peak initial light are believed similar, the temporal occurrence of the two signals may be less so; for example, there is evidence that Ca^{2+} entry promotes rapid repolarization (*14*), an event that is nearly over before luminescence is detectable. An alternative hypothesis, that the initial phase represents predominantly release of Ca^{2+} from stores, cannot at present be excluded, but this would require that such release has a simple interval dependence similar to that of I_{si} rather than the more complex interval dependence usually attributed to release of Ca^{2+} from internal stores (*4*).

The decline in light that may immediately follow the initial rapid phase cannot be interpreted unequivocally. It certainly represents a decrease in [Ca^{2+}] but this decrease could be due to redistribution by diffusion, as well as active uptake into an aequorin-free compartment, or passive binding.

After the initial rapid rise, the time course and peak amplitude of the aequorin signal are not related in a simple way to the interval between stimuli. The signal may decline monophasically, it may have a plateau, or continue to rise to a peak before declining. The first case is usually evident only in depressed inotropic states (extrasystole, Fig. 2B), the second may occur during steady stimulation at regular intervals, and the last also occurs during steady stimulation at long intervals, but occurs particularly in potentiated inotropic states (Fig. 2B). Thus, this part of the aequorin signal is increased in potentiated inotropic states and decreased in depressed inotropic states. However, a direct relation between peak light intensity and peak force does not exist. The dependence of this part of the aequorin signal on the stimulus interval and the pattern of stimulation is not similar to any described interval dependence of I_{si}. This raises the possibility that an underlying process is release of Ca^{2+} from intracellular storage sites. This interpretation would be consistent with numerous studies that have implicated increased release of Ca^{2+} from storage sites as the major cause of inotropic effects produced by certain changes in the pattern of stimulation, for example, paired stimulation (*4*) (compare Fig. 2B).

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12. For an experiment in which a single cell was injected with aequorin and 700 responses were averaged, the minimum detectable L/L_{max} (L is light intensity during the response, and L_{max} is peak light intensity in saturating [Ca^{2+}] calculated from total light emitted during lysis of the cell) was 1.3×10^{-6}. This would correspond to $10^{-7}M$ Ca^{2+} for aequorin at 37°C, in the presence of 2 mM Mg^{2+} and 150 mM KCl. This is believed to be comparable to the more typical experimental situation in which ten cells were injected and 64 to 128 responses were averaged.
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17 September 1979; revised 12 December 1979

Parathyroid Hormone: A Coronary Artery Vasodilator

Abstract. *Injection of synthetic bovine parathyroid hormone (the amino terminal peptide containing the first 34 amino acids) to the coronary circulation of the dog resulted in a marked coronary vasodilation. The vasodilatory response was dose-dependent and amounted to a 161 percent increase over the resting flow rate at a concentration of 1.0 unit per kilogram.*

A synthetic preparation of bovine parathyroid hormone containing the first 34 amino acids [bPTH(1-34)] was tested for possible vasoactive effects on the coronary circulation and other cardiovascular responses in the dog.

Five mongrel dogs of either sex and weighing 19 to 38 kg were used. The dogs were anesthetized with sodium pentobarbital (30 mg/kg), tracheostomized, and ventilated with room air by means of a positive pressure respirator (Harvard Apparatus). A left thoracotomy was performed through the fifth intercostal space. The pericardium was incised and sutured to the chest wall to form a cradle for the heart. A length (2 cm) of the left circumflex coronary artery was freed of surrounding connective tissue and a 7- to 9-mm electromagnetic flow probe was placed on the artery. The flow rate in the left circumflex artery was determined by means of a Carolina model 501D electromagnetic flowmeter (Carolina Medical Electronics). A 23-gauge thin-wall vein needle tip was inserted into the artery distal to the flow probe and allowed to remain indwelling during the course of the experiment for bPTH(1-34) or isotonic saline (control) injections.

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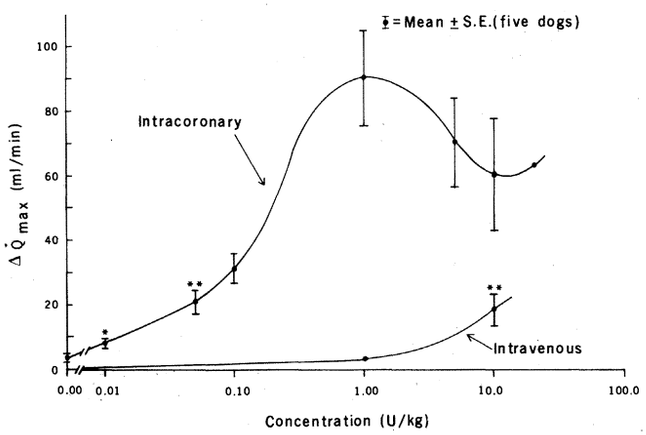

Fig. 1. Effects of bPTH(1-34) on coronary flow rate. Isotonic saline or bPTH 1-34) in varying doses was injected into the left circumflex artery (intracoronary) or right femoral vein (intravenous). Each point represents the mean maximal change in flow rate ($\Delta\dot{Q}_{max}$) in five dogs at a given concentration of bPTH(1-34). One unit of bPTH(1-34) is equivalent to 100 ng. Symbols: *, $P < .05$ and **, $P < .001$, compared to saline control.

To monitor myocardial contractile force in the region of the left circumflex blood flow we attached an adjustable Walton-Brodie strain gauge arch to the posterolateral wall of the left ventricle. Catheters were placed in the right femoral artery and vein for arterial blood pressure recording (via a Statham P23 AC pressure transducer) and bPTH(1-34) injection or anesthesia supplementation, respectively. Electrodes were placed on each of the four limbs for recording electrocardiogram (EKG) (lead II) changes and heart rate. Left circumflex artery flow, arterial pressures, contractile force, and EKG were monitored simultaneously on a four-channel Grass model 79 recorder. Estimated mean arterial pressure ($\bar{P}_a$) was calculated from the recorded systolic (P_s) and diastolic (P_d) arterial pressures.

Synthetic bPTH(1-34) was obtained from Peninsula Laboratories and contained 10,000 IU/mg according to the assay in vitro for adenyl cyclase activation in the rat renal cortex. According to Peninsula Laboratories, the bPTH(1-34) is pure. Only one spot can be detected by thin-layer chromatography with two different solvent systems and on high-voltage electrophoresis. For injection, the bPTH(1-34) was dissolved in isotonic saline. Statistical analyses (means, standard error of the means, and Student's *t*-test) were performed on a Texas Instruments 59 programmable calculator.

Injection of bPTH(1-34) resulted in a marked vasodilation that was dose-dependent over the range of 0.01 to 1.0 U/kg. Figure 1 shows the maximum change in flow rate ($\Delta\dot{Q}_{max}$) after intracoronary administration of bPTH at each concentration tested. The greatest flow response was obtained with 1.0 U/kg. The $\Delta\dot{Q}_{max}$ increased from 8.0 ± 1.1 ml/min with 0.01 U/kg, the lowest concentration tested, to 89.9 ± 14.1 ml/min after the administration of 1.0 U/kg. The peak change in recorded circumflex flow rate after the administration of 1.0 U/kg (137.0 ± 15.9 ml/min) represented a 161 percent increase over flow rates before the injection (52.5 ± 3.7 ml/min).

The time of onset of the $\dot{Q}$ response after intracoronary injection did not vary significantly at any bPTH(1-34) concentration (that is, 8 ± 3 seconds with 0.01 U/kg and 9 ± 1 seconds with 1.0 U/kg)

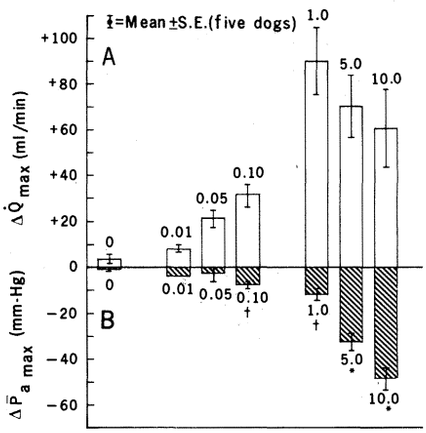

Fig. 2. Changes in coronary flow rate and mean arterial pressure after injection of isotonic saline or various doses (indicated above or below each bar) of bPTH(1-34) into the left circumflex coronary artery. In (A) each bar represents the mean maximal change in flow rate ($\Delta\dot{Q}_{max}$) in five dogs at the bPTH(1-34) concentration indicated. In (B) each bar represents the mean maximal change in mean arterial pressure ($\bar{P}_a$) in the same five dogs at the bPTH(1-34) concentration indicated. Mean arterial pressure was estimated from the following: $\bar{P}_a = (P_s + 2P_d)/3$, where P_s and P_d are arterial systolic and diastolic pressures, respectively. One unit of bPTH(1-34) is equivalent to 100 ng. Symbols: *, $P < .001$ and †, $P > .1$, compared to the 0.05 U/kg dosage.

($P > .2$). Time to maximum flow also did not differ significantly ($P > .1$). Duration of the vasodilatory response increased significantly with bPTH(1-34) concentration. Duration of the vasodilation was 162 ± 46 and 797 ± 103 seconds at 0.01 and 1.0 U/kg, respectively ($P < .01$). Tachyphylaxis to successive intracoronary injections of bPTH(1-34) was not observed.

Intravenous injection of bPTH(1-34) (10 U/kg) similarly resulted in coronary vasodilation; the response being, as expected, quantitatively less compared to intracoronary injection (10 U/kg) because of hemodilution. Since the mean body weight of the dogs was 24.7 ± 2.4 kg, and if one assumes a plasma volume of 1.1 liters, the $\Delta\dot{Q}_{max}$ with an intravenous injection of 10 U/kg should have been approximately equivalent to that of an intracoronary injection of 0.01 U/kg or less. Indeed, the mean responses were not found to be different ($P > .05$). Doses of bPTH(1-34) greater than 10 U/kg were not tested by the intravenous route.

Time of onset of the coronary vasodilatory response was similar with both modes of injection. A reduction in peripheral vascular resistance clearly resulted also from bPTH(1-34) injection. A decrease in $\bar{P}_a$ was observed with intracoronary doses of 0.01 U/kg and greater. The $\bar{P}_a$ response was dose-dependent over the range of 0.10 to 10 U/kg, with the maximum response occurring at the highest dose tested, 10.0 U/kg. The hypotensive response was reflected in the depression of $\bar{P}_a$ (Fig. 2) during the period of maximal coronary flow, an observation that rules out, directionally, the possibility that increased coronary perfusion pressure caused the increased flow. Indeed, the observed decreases in $\bar{P}_a$ would have tended to modulate the vasodilatory response. Thus, it is likely that the coronary flow response to bPTH(1-34) arose directly from a decrease in coronary vascular resistance. The coronary vasodilation and hypotensive responses were not accompanied by a change in heart rate (that is, we observed no chronotropic response). However, an increase in contractile force was observed after both intracoronary and intravenous bPTH(1-34) injection. The increase in contractile force (up to 46 percent compared to control) was dose-dependent over the range of 0.1 to 5.0 U/kg.

The effects of parathyroid hormone on vascular tissue have received little investigation; to our knowledge this is the first study with respect to the coronary circu-

lation. Earlier work by Charbon [see (*1*, *2*)] showed that intravenous injections of parathyroid extract increased blood flow in the coeliac and renal circulations of the dog. More recently, Charbon and Hulstaert (*3*) demonstrated that the Beckman synthetic bPTH(1–34) peptide, possessing a lower activity (2846 IU/mg), could cause a four- to fivefold increase in hepatic artery blood flow. Thus the bPTH(1–34) vasodilatory response is not limited to the coronary circulation. The hypotensive responses elicited by bPTH(1–34) in the present studies implied a generalized vasodilatory effect. Also, bPTH(1–34) has been shown to increase blood flow rate in dog kidneys and rat hind limbs and to relax helical strips of rabbit aorta (*4*). Of interest has been the inability to block the blood pressure-lowering effect of bPTH(1–34) in dogs and rats with α- or β-adrenergic, cholinergic, or histaminergic blocking drugs (*4*). This suggests to us that the coronary vasodilatory response to synthetic bPTH(1–34) is probably a direct one, involving specialized receptors on the vascular smooth musculature.

The vasodepressor response to bPTH(1–34) has been demonstrated in a variety of species and preparations by Pang *et al.* (*4*), including South American lungfish, bullfrog, water snake, domestic chicken, and rat.

Our findings with a pure synthetic form of a naturally occurring polypeptide hormone may be useful in understanding the regulation of coronary smooth muscle tone. The fact that a known primary function of parathyroid hormone is the regulation of blood calcium level, coupled with the well-known importance of calcium in the control of vascular smooth muscle contractility, lends further significance to the present observations.

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26 December 1979

Identification of Mosquitoes of *Anopheles gambiae* Species Complex A and B by Analysis of Cuticular Components

Abstract. *Two important vectors of malaria in Africa,* Anopheles gambiae *and* Anopheles arabiensis *(Diptera: Culicidae), often occur sympatrically and cannot be distinguished morphologically. A chemical method was developed to identify individual laboratory-reared adult males or females of either species by extraction and analysis of cuticular components with gas chromatography. Statistically significant differences were seen between species when selected pairs of peaks were compared.*

The *Anopheles gambiae* Giles complex of Africa includes two species that breed in salt water, namely *An. melas* Theobald in West Africa and *An. merus* Donitz in East Africa. Three more species, which breed in freshwater, were previously designated as species A, B, and C and are now designated *An. gambiae* Giles, *An. arabiensis* Patton, and *An. quadriannulatus* Theobald (*1*, *2*), respectively. A fourth member, species D (Bwamba cytotype), is found only in waters with high mineral contents in the Semliki forest of Uganda (*3*). The two most important species, *An. gambiae* (A) and *An. arabiensis* (B), are highly anthropophilic, are the principal vectors of malaria in the Afrotropical region, and occur sympatrically in many areas. They are true sibling species in which interbreeding gives sterile males, and they can only be separated with confidence by the banding of the polytene chromosomes of either the fourth-instar larval salivary glands (*4*) or the adult ovarian nurse cells of half-gravid females (*5*). Recently, however, a method has been described for identifying larvae and pupae of these two species by fluorochrome bisbenzimid staining of the mitotic karyotypes (*6*). In another procedure that has been tried, diagnostic allozymes are used for separating certain species of the *gambiae* complex (*7*, *8*).

The usefulness of these methods for work in the field requires further evaluation because each has severe technical limitations. For example, only females can be identified by chromosomal studies, and they must be half-gravid. In addition, cytotaxonomic identifications are difficult on preserved material with the result that many individuals cannot be identified (*9*). For allozyme studies it is necessary to keep field-collected samples continuously frozen in Dry Ice from time of collection until laboratory analysis.

We therefore investigated alternative methods of identifying adults of either sex of *An. gambiae* and *An. arabiensis*. Extraction and analysis of chemically stable and easily obtained cuticular hydrocarbons was carried out by gas chromatography (GC) to discover whether there were specific ingredients or unique relationships that could serve as markers. We report here some minor but statistically significant differences in the cuticular components of laboratory-reared adults of these two sibling species.

Sample of 2- to 3-week-old unmated mosquitoes were obtained from laboratory colonies of *An. gambiae* and *An. arabiensis* maintained at the Liverpool School of Tropical Medicine in England.

Crude lipids were extracted from cuticular samples of individuals or pooled samples of each sex and species by soaking each sample twice in a minimum of *n*-heptane. The extract was transferred to a clean glass vial, and the solvent was removed for shipment by evaporation with a stream of dry air. Crude extracts were redissolved in a minimum amount of hexane (10 μl) for immediate GC analysis (Fig. 1) or added to a column prepared from a disposable Pasteur pipette (5 mm in diameter), dry-packed with 60 to 200 mesh silica gel (2 cm) over 20 percent silver nitrate–impregnated silica gel (1 cm). Saturated paraffins were eluted with *n*-hexane, olefins with 1 percent ether in hexane, and polyolefins with 15 percent ether in hexane. The volume of each separated sample was adjusted to 10 μl for GC analysis (*10*).

The major constituents of the crude lipid extract were *n*-paraffins of 21 to 33 carbons and branched paraffins of 29 to 44 carbons, consistent with a previous analysis of pooled samples of 500 mosquitoes (*11*). Patterns in the chromatograms of components with 21 to 44 carbons appeared to be similar in all samples. Also in this range were smaller peaks, but none that appeared to be unique to either species or either sex. Saturated or unsaturated hydrocarbons that might be species markers were not found in sufficient quantities to be useful.

Quantitative data on the distribution of larger peaks suggests that there are at least three relationships that can be used for identification markers of females of

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