Bradykinin as a Mediator of Human Pain.* (32489)

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It has been shown that bradykinin causes pain in man when applied intradermally(1, 2), intraarterially(3), intraperitoneally(4) or to a blister base(5,6). It has also been demonstrated that bradykinin is one of the most potent pain-producing substances(7). Lim (8) has stated that pain is essentially a chemical sensation and has used the response to bradykinin as an example for his theory. It is, therefore, possible to hypothesize that this peptide acts as one of the general mediators of pain caused by noxious stimuli.

The nonapeptide bradykinin and the decapeptide kallidin are apparently formed in many, but not all, inflammatory exudates(7). Their pharmacological properties are strikingly similar and include bronchoconstriction in the guinea pig, increased capillary permeability, vasodilatation, accumulation and migration of leucocytes, and causation of pain and hyperalgesia. The endecapeptide eledoisin shares many of these qualitative properties with bradykinin and kallidin, but frequently differs from them quantitatively, and its chemical structure is dissimilar(9).

We have been interested in the experimental production of deep somatic pain and have developed a standard psychophysical technique using intramuscular injections of hypertonic sodium chloride solutions(10). In view of the experimental work reported by other workers with bradykinin demonstrating its potent pain-producing properties(7), we decided to substitute this and similar polypeptides for saline. To our surprise, no pain was produced intramuscularly with any concentration of bradykinin (up to 2 mg) in any of our initial group of 6 healthy volunteers using our standard deep somatic pain-producing technique. Therefore, we decided to examine the alleged pain-producing properties of bradykinin (and the related polypeptides, kallidin and eledoisin) systematically.

Materials and methods. Twelve healthy, paid volunteers (5 males and 7 females), age range from 22 to 37 years, were selected and screened for the absence of any previous abdominal surgical procedures. All subjects participated in the intramuscular injections, which were made following our standard technique(10). Briefly, this consists of inserting 32 25G 2 in hypodermic needles in rosette fashion into both gluteus medii muscles through 8 superficial blebs containing 1% procaine hydrochloride, 4 needles per bleb. The tips of the needles are separated approximately 1 inch from each other within the muscle. Our standard method involves slow injections of saline in ascending and descending series of concentrations, ranging from isotonicity to a possible maximum of 10.0% NaCl w/v, in steps of 0.5% NaCl in constant volumes of 0.2 ml at 2 minute intervals. In this experiment each subject was given various concentrations of hypertonic NaCl, sterile water, and bradykinin, all in quantities of 0.2 ml, using a single-blind approach. Prior to each experiment every subject was instructed to report "pain" whenever he experienced any kind of pain, ache or hurting sensation. The latency of onset and duration of each pain response was timed. All subjects reported pain following pure water injections, and also to hypertonic saline stimulation, varying in concentration from 2.0 to 5.0% NaCl. Bradykinin in concentrations up to 2 mg never produced pain in any of our subjects (Table I). This latter bradykinin concentration is about 10^2 to 10^4 greater than those used by other investigators for human stimulation by other routes.

Six of our subjects also volunteered to have intraperitoneal stimulation. (These 6 subjects on the intramuscular administration received kallidin and eledoisin in addition to

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bradykinin, hypertonic saline and pure water). For the intraperitoneal injections, 50 cm of Clay-Adams PE 50 polyethylene tubing was threaded into the peritoneal cavity through a long 18G needle after anesthetizing the skin and muscle layers. This represented a slight modification of Lim's(4) method. A 3-way stop cock was attached to the exterior tubing by means of a metal adapter. A saline infusion was placed in the side arm and test injections were made by syringe through the third opening. After each experimental injection 5 drops of normal saline were allowed to run through the tubing. All 6 subjects received single-blind injections of bradykinin, hypertonic saline, and pure water, in volumes from 0.05 to 0.1 ml. In addition, 5 subjects also received the two other polypeptides, kallidin and eledoisin.

The last 4 subjects were also given subcutaneous injections of these 3 polypeptides as well as of hypertonic saline and distilled water in volumes of 0.1 ml. These subcutaneous injections were made in an unanesthetized portion of the skin overlaying the gluteus medius muscle.

Results. It will be noted (Table I) that both bradykinin and kallidin produced pain in minute dosages on intraperitoneal injections. Eledoisin did not. None of the kinins produced pain on intramuscular or subcutaneous injections in very much higher dosages. The only exception is kallidin which produced a weak pain of short duration in one subject on intramuscular injection of 50 μg. The control substances, *i.e.*, distilled water and hypertonic saline, produced pain by any of the 3 routes of injection, although of much weaker intensity and duration intraperitoneally than the kinins.

The vehicles for the 3 polypeptides ranged in pH from 4.6 to 5.0, and consisted of a glacial acetic acid-sodium acetate buffer with added chlorobutanol, sodium chloride and distilled water. The vehicle by itself usually caused an immediate and very short lived (about 3 to 5 sec) pain response intraperitoneally. This could be clearly distinguished from the long latency of onset (averaging 25 sec) and duration (averaging 20 min) of the pain response to the active compounds intraperitoneally. The vehicle caused little or no pain on subcutaneous or intramuscular injections.

The last 4 subjects were also stimulated intradermally, but we were unable to evaluate intradermal effects since the vehicle alone given by this route caused as much pain as the polypeptides. Furthermore, all intradermal pain responses were of very short duration (1 to 10 sec), thus not permitting evaluation in terms of time. Therefore, intradermal results have not been included in Table I.

Discussion. These findings are incompatible with the concept that these polypeptides, bradykinin, kallidin and eledoisin, act as universal mediators of pain. On the other hand, pain can easily be produced by intramuscular, subcutaneous or intradermal injections of a variety of substances, such as hypertonic or hypotonic sodium chloride, potas-

TABLE I. Minimal Pain-Producing Doses of Bradykinin, Kallidin and Eledoisin with Intraperitoneal, Subcutaneous and Intramuscular Injections. Abbreviation: N, No. of Subjects.

	Bradykinin	Kallidin	Eledoisin
	(μg)		
Intraperitoneal	N = 6 median = 1.0 range = 0.2-15.0	N = 5 median = 2.8 range = 0.5-50.0	N = 5 No response up to 17.5
Subcutaneous	N = 4 No response up to 50.0	N = 4 No response up to 50.0	N = 4 No response up to 50.0
Intramuscular	N = 12 No response up to 2000.0	N = 6 No response up to 70.0 (except 50.0 for one subject)	N = 6 No response up to 50.0

sium chloride, histamine, etc.(7,10,11,12). Therefore, either one must postulate some different mechanism for pain mediation or these polypeptides are only one of a number of mediators with perhaps special activity in endothelial or mesothelial-lined structures.

Summary. In man, the polypeptides bradykinin and kallidin, but not eledoisin, produce pain on intraperitoneal injection. None of these substances produces pain when injected subcutaneously or intramuscularly in higher concentrations. Therefore, none of these polypeptides can be considered as a universal mediator of pain.

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Heart Rate of the Developing Chick Embryo.* (32490)

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The two common techniques used to measure heart rate of avian embryos require removing a portion of the shell to form an observation window(1,3) or implanting electrodes within the egg(4-6). These procedures expose the embryo to shock, injury and infection. In the latter case there can be tissue reaction to the electrodes, which may be further aggravated by frequent embryonic movements causing changes in position of the implanted probes. Temperature also influences the frequency of embryonic heartbeat; it has been reported that 0.5°C fluctuations were sufficient to cause considerable rate changes(4). In order to determine a "normal" embryonic heart rate, a method should be employed which does not require penetration of the shell or other disturbance to the embryo and which allows maintenance of the normal incubation environment.

A miniature ballistocardiograph developed at the Ames Research Center(7,8) provides a method by which motions within a living organism can be monitored with a minimum of disturbance to it. This instrument is an ultra-sensitive piezoelectric transducer which utilizes some of the basic principles and components of a micrometeoroid momentum transducer(9), but is especially designed for specific application as a high frequency ballistocardiograph for avian embryos. The device measures the acceleration of the egg shell induced by embryonic movement, especially heart beat, and incorporates electrical and mechanical common-mode noise rejection features, The object of the present study was to compare heart rates of developing chick

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