EFFECT OF BRADYKININ AND ITS ANTAGONISTS ON SUPERFICIAL AND DEEP PAIN IN HUMAN SUBJECTS AND DOGS

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It has been demonstrated that release of kinins like bradykinin and kallikrin during tissue injury is responsicble for local vasodilatation, increased capillary permeability, diapedisis of leucocyte and pain (1) . Further aspirin like compounds are known to antagonize the effects of kinins which may be responsible for their antiinflammatory activity (2). Similarly an antagonism of bradykinin evoked pain by narcotic and non-narocotic analgesics has been reported by Lim *et al* (6).

Therefore it was planned to study the effects of antibradykinin compounds on superficial and deep pain produced by the application of *liquor epispasticus* and bradykinin on blister base in human beings and by injecting (intraperitoneally, subcutaneously and intra- arterially) in dogs respectively. The pain producing effect of bradykinin and its antagonists on human blister base was also studied in neural leprosy and compared with normal human subjects.

MATERIALS AND METHODS

Blisters were produced on the flexor surface of the fore arm, of human subjects, through application of *liquor epispasticus* a day before the commencement of the experiments. The skin of the blister was cut a-septically and its base was washed with Ringer's solution. All experimental drugs were dissolved in normal saline and kept at 37° C temperature in an incubator. Each drug solution in varying concentration (Table I) was applied to the blister base area with a sterile glass dropper till the area was filled. Each drug was allowed to act till pain reached a platue or began to subside, up to a maximum of two minutes. The drugs were applied at intervals of 10, 20, 30, 40, 50 and 60 minutes. The subject was kept ignorant of the nature of the solution applied and he himself assessed the pain intensity of each solution subjectively. For the test of cutaneous pain, the drugs employed are given in Table I. The experiments were conducted on twenty normal human subjects and equal number of neural leprosy patients. Twenty cases of neural leprosy were selected for the study. The history of the patients revealed that the duration of the disease ranged between 1 to 15 years. All cases selected had involvement of the ulnar nerve. These cases had thickening of the ulnar nerve and on the basis of the degree of nerve thickening they were divided into three categories. Cases of mild thickening were 5, moderate thickening 7 and greatly thickened 8 cases. The patches of anaesthesia varied from 1″ to 3″ in diameter only. Similarly the sensory loss in the patches of anaesthesia was assessed as mild* moderate** and severe***.

TABLE I

Drugs Used	Dosage	Mode of administration
Histamine	0.1 to 500/$\mu g/ml$	Applied locally on blister base area
*Bradykinin	0.1 to 1000/$\mu g/ml$	
5-HT	0.1 to 100 /$\mu g/ml$	

*Bradykinin and Histamine administered intradermally also.

Acetylcholine	10 to 100/μg/*ml*	
Hcl+Bradykinin	10/μg/*ml* bradykinin	
$NaHCO_3$	1.5%, 5 *ml*	Local application
Acetyl Salicylate	3 *mg/ml*	Local application
Emetine	0.66 *mg/ml*	Do
Morphia	5-10 *mg/kg/wt* 30 minutes before bradykinin	Sub-cutaneously
Codein	40 *mg/kg/wt* 30 minutes before bradykin.	Do.

Pain assessment, mild+Moderate++and severe+++
Absence of pain; mild—moderate– – and complete– –

In a second series of experiments on deep (visceral) pain, the following drugs were used on ten lightly anaesthesized dogs. TABLE II (Chloralose, 30 *mg/kg* I.V.)

TABLE II

Drugs Used	Dosage	Mode of Administration
1. Bradykinin	100/μg/*ml*	sub-cutaneously
2. Bradykinin	Do	Do
3. Amidopyrine	160 *mg/kg*/10 *ml.*	Intra-peritoneally
4. Acetyl-salicylate	200-500 *mg/kg*/10 *ml*	Intra-peritoneally
5. Do		Do
6. Bradykinin	0.1 μg/*ml*	Intra-arterially
7. 5 HT	Do	Do
8. Acetyl-choline	Do	Do
9. Codein	40 *mg/kg*/wt	Intra-peritoneally in 10 *ml*
10. Codein (30 minutes after bradykinin (100/*ug*/5 *ml*)	Do	Do

Two cases of human scorpion stings, were studied and the effect of 5 ml. of 2% Novocaine and emetine, 1 *gr*/5 ml saline injected locally at the site and the neighbouring area was studied seperately.

The minimal dose of some drugs required to produce pain, has also been studied (Table III).

MINIMAL DOSAGE OF SOME DRUGS ELICITING PAIN IN HUMAN BEINGS.
TABLE III

Drugs Used	Dosage in μg/*ml*	Human Subjects	
		Normal	Neural Leprosy
1 Bradykinin	0.1 to 10	+	..
2 Acetyl-choline	10 to 100	+	..
3 Histamine	0.1 to 100	+	–
4 5-Hydroxytroptamine	0.1 to 100	+	..

RESULTS

As a result of intra-dermal injection of bradykinin, an increase in the volume of the bleb was noticed and this was associated with slight erythrema in four cases. This observation was in marked contrast to the cutaneous vascular response produced with histamine in the same way. When bradykinin (2 $\mu g/ml.$) was brought in contact with the nerve endings in the dermal layers of the skin, it produced severe pain. When 10 to 20 μg dose were employed, it caused itching, flare and wheel besides the pain. The threshold level of bradykinin required to produce pain by this method was found to be 10 $\mu g/ml$ and the effect lasted for 30 to 60 seconds. Tachyphylaxis developed after repeated appilcations. Histamine was found to be active at a threshold level of 10^{-6} $\mu g/ml.$ It caused itching, pricking and pain which lasted for two to three minutes and no tachyphylaxis developed.

When bradykinin and N/50 HCL were applied together, no tachyphylaxis developed. But application of 1.5% $NaHCo_3$ and emetine (1 gr. solution) after bradykinin, reduced pain considerably. When acetylcholine was applied, it produced mild pain. Intradermal injection of 5. μg of bradykinin produced a flare of 5 cm. which lasted for 5-10 minutes only.

There was no appreciable effect of bradykinin application on neural leprosy patients (Table III)

The pain of scorpion sting was markedly reduced after emetine solution infiltration at the site of the sting and its neighbouring area. Infiltration of the area with 5 ml of 2% novocaine solution produced mild depression of the pain and this re-occured after few minutes.

The experiments on dogs, showed that codein suppressed nociception response in dogs. Amidopyrine and acid salicylate administration after bradykinin, did not affect pain appreciably. The intra-arterial injection of bradykinin, 5-hydroxytryptamine and acetylcholine resulted in loud and persistent vocalization of the experimental dogs. The moderate vocalization during anaesthesia was compared with that of visceral pain vocalization.

DISCUSSION

After application of various drugs (Table I) ,no tachyphylaxis developed except in the case of bradykinin repeated application. The incidence of tachyphylaxis appears to depend on the mode of kinin formation. It is possible that the kinin mediator may be a slightly different peptide from the synthetic non peptide in human beings.

In the case of neural leprosy subjects, it was observed that application of drugs (Table III) did not effect the pain receptor. This could be due to the fact that mediator of inflamation does

not itslef cause the pain but lowers the threshold for firing off afferent impulses and renders the area hyperalgesic to otherwise threshold stimuli. The pain fibres are present in the afferent sensory nerves and the sensory nerves are damaged to a varying extent by fibrosis and the pain sensations are lost according to the degree and extent of damage in neural leprosy.

The application of drugs on blister base in normal human subjects (Table I) produced pain. This production of the pain could be due to the fact that body is rendered hyperalgesic by liquor epispasticus (which contains bradykinin and allied kinin) and the pain threshold is lowered in the regions of the flare induced by noxious stimulation of the skin. This raises the possibility of vaso-dilator mediating substances to be associated with the flare response of the skin in noxious stimulation and has the property of lowering the thresh-hold for activating the neurones subserving the pain sensation. The above evidence indicates the release of humoral substance as a result of neural activity evoked by noxious stimulation and even possibility of bradykinin implicating the reaction.

The pain producing effect of histamine on blister base could be due to the release of bradykinin in the interstitial fluid where it comes in contact with the activator from injured cells to form bradykinkn and hence it mediates in the inflamatory response and produ tion of the pain.

The role of bradykinin antagonists (Table I) in the diminution of pain seems to depend on the ability of the agents to inactivate bradykinin at the site (2). The other possibility could be due to neural activity involving various levels of integration, including the highest which modify reactions to noxious stimulation in the peripheral tissues in such a way as to augment or to suppress inflammation and tissue damage. The liberation or accumulation of humoral mediators in the periphery is implicated in these reactions (3).

In the study of scorpion sting pain, it was found that infiltration of 2% novocaine (in 5 ml saline) diminshed pain intensity but this lasted for 5 to 10 minutes and re-occurred. Probabably this effect is due to competitive antagonism of novocaine with bradykinin. The local infiltration of emetine solution diminished the pain to a great extent and it did not reoccur. This could be due to the fact that probably emetine degrades bradykinin and hence diminshes the pain.

In a series of experiments on dogs (Table II), it was observed that acetyl salicylate is effective against visceral pain receptors unlike skin nociception which was not affected by amidopyrine or calcium acetyl salicylate. The intra-arterial injection of bradykinin, acetyl-choline and 5-hydroxy tryptamine in lightly anaesthetized dogs resulted in vocalization. These effects are akin to that of pseudo-effective responses described by Wood Worth (7) and were similar to painful stimuli in intact animals This similarity of response is strongly suggestive of the fact that intra-arterial injection of bradykinin produced pain but more in comparisoon to acetyl-choline which produced only mild pain. 5-hydroxytryptamine was found to be more potent than bradykinin on visceral receptors. The present finginds are quite in conformity with Chapman and Goodell (4).

Lim (5) showed that intra-arterial injection of bradykinin in various viscera (brain, heart and knee joints) of animals resulted in vocalization which was due to stimulation of chemo-receptors which are quite different from mechano-receptors or thermo-receptors. They further thought that these were afferent sensory nerves which do not end in capillaries but in interstitial connective tissue spaces and were not vasomotor nerves.

Lim (6) further demonstrated, visceral pain producing effect of bradykinin which was blocked by non narcotic analgesic (sodium acetyl salicylate., I.V. in therapeutic dose). The vocalization was due to visceral pain and not the cutaneous one and this pain could be produced with a /μg or even less of bradykinin dose.

Conclusions

Bradykinin and 5-hydroxytryptamine are more painful in comparison to histamine and acetyl-choline on human blister base area.

Cutaneous pain receptors are antagonized by morphine and codein and visecral by acetylc salicylic acid.

Bradykinin and HCL combined application produced less tachyphylaxis than bradykinin alone.

Novocaine and emetine diminished scorpion sting pain.

Intra-arterial injection of bradykinin, 5 HT and acetyl-choline casued vocalization (Visceral pain) in dogs.

REFERENCES

1. Elliott D.F., E.W. Horton; and A.O. Goodell. Action of pure Bradykinin. *J. Physiol.* **153:** 473, 1960.

2. George, E., B. Nicholas; P. Depasquale. Bradykinin. *Am. Heart. J.* **65 :** 116, 1963.

3. Loring, F., C. Armando; D., Helen Goodell B. D. and Harold G. Wolff. Evidence for kinin formation from neural activity evoked by noxious stiumlation. *Ann. of N. Y. Acad. Sci.* **104** : 269. 1963.

4. Loring, F. C. Armando, D. Ramos Helen, B.D. Goodell, and Harold G. Wolff. Neuro humoral features of afferent fibres in man—their role in vasodilation, inflamamation and pain. *Arch. of Neurolo.* **4** . 617 1961.

5. Robert, K.S; Lim, Chaunao Liu Franck Guzman & Christian Braun. Visceral receptors concerned in visceral pain and the pseudo-effective response to intra-arterial injection by bradykinin and other algesic agents *Comp. Neurol.* **48 ;** 229. 1962.

6. Robert, K.S., Lim, F. Guzman; D.W. Rodgers; K. Goto; C. Braun G.D., Dickerson and R.J. Eagle. Site of action of narcotic and non-narcotic analgesics determined by blocking bradykinin evoked visceral pain. *Arch. Int. Pharmacodyn. Ther.* **152** : 28 1964.

7. Wood Worth, R.S. & C.S. Sherrington. A pseudo-effective reflex and its spinal-path *J. Physiol.* **31 :** 234, 1904.