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Bradykinin-induced knee joint incapacitation involves bradykinin B_2 receptor mediated hyperalgesia and bradykinin B_1 receptor-mediated nociception

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Abstract

The participation of B_1 and B_2 types of bradykinin receptors was studied in the rat knee-joint incapacitation test. Five intra-articular successive hourly administrations of bradykinin produced progressive incapacitation, thus indicating that bradykinin induced sensitization to its own nociceptive effect. Four co-injections of bradykinin with the bradykinin B_1 receptor antagonist des-Arg^9-[Leu^8]bradykinin were without nociceptive effect. However, a 5th injection of bradykinin alone produced intense incapacitation. The bradykinin B_2 receptor antagonist HOE-140 ([D-Arg^0[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin), or indomethacin, prevented the bradykinin-induced incapacitation. However, successive co-injections of bradykinin with prostaglandin E_2, in contrast to bradykinin alone, did induce incapacitation in animals pretreated with indomethacin or HOE-140. The injection of the bradykinin B_1 receptor agonist des-Arg^9-bradykinin into prostaglandin E_2-treated joints did induce incapacitation, although administration of the bradykinin B_1 receptor agonist or prostaglandin E_2 alone did not induce incapacitation. In conclusion, in ongoing articular inflammation, it is suggested that the bradykinin B_1 receptor is particularly involved with nociceptor activation, while the bradykinin B_2 receptor is related to nociceptor sensitization.

Keywords: Inflammation; Arthritis; Prostaglandin E_2; HOE-140; Des-Arg^9-bradykinin; (Rat)

1. Introduction

Subcutaneous infusion of bradykinin has been long to cause overt pain in humans (Armstrong et al., 1957; Sicuteri et al., 1965; Ferreira, 1972). But, in addition, it has also been reported that in some experimental models, bradykinin causes a delayed and long-lasting hyperalgesic effect. This hyperalgesic effect seems to be mediated by the release of prostanoids, since it was inhibited by the cyclooxygenase inhibitor indomethacin, but it was not necessarily associated with overt pain (Taiwo and Levine, 1988; Steranka et al., 1987, 1988).

Numerous electrophysiological studies associated bradykinin B_2 receptors with direct activation of fine afferent fibres which are thought to conduct stimuli associated with nociception (see Steranka and Burch, 1991; Farmer and Burch, 1992). The bradykinin B_2 receptors have also been associated with direct stimulation of cutaneous pain receptors in man (Whalley et al., 1987). Nevertheless, bradykinin B_2 receptors were shown to mediate nociceptor inflammatory sensitization in the rat and mouse (Beresford and Birch, 1992). This sensitization has recently been shown, with the use of specific cytokine antibodies, to be mediated by hyperalgesic cyclooxygenase products provoked by bradykinin release of tumour necrosis factor-α and interleukin-1 (Ferreira et al., 1993). Thus, the bradykinin B_2 receptor has been associated with nociception and hyperalgesia.

On the other hand, recent studies suggest that bradykinin B_1 receptors also mediate ongoing hyperalgesia in rats (Perkins et al., 1993; Davis and Perkins, 1994). Furthermore, bradykinin B_1 receptor antagonists had been shown previously to inhibit the immediate nociceptive response to the formalin test in mice (Shibata et al., 1989; Corrêa and Calixto, 1993), suggesting that the bradykinin B_1 receptor may also be involved in overt nociception. A major problem for the evaluation of which type of bradykinin recep-

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tor is involved in the sensitization or activation of the nociceptors may arise when both parameters are not studied in parallel in the same behavioural test.

The aim of the present work was study the bradykinin B_1 and B_2 receptor involvement in the rat knee-joint incapacitation test. This model of acute arthritis permits discrimination between sensitization (hyperalgesia) and activation of the nociceptors as evidenced by the presence of overt behaviour (incapacitation).

2. Materials and methods

2.1. Animals

Experiments were performed on male Wistar rats (150–180 g) which were housed under a 12-12 h light/dark cycle with free access to water and food.

2.2. Algesimetric test

The rat knee-joint incapacitation test is described in detail elsewhere (Tonussi and Ferreira, 1992). In this test, a computer-assisted device measures the time that a specific hind paw fails to touch the surface of a rotating cylinder over a 1-min period (paw elevation time). Normal, control, paw elevation time is approximately 10 s. In our experiment, incapacitation was quantified as an increase in the paw elevation time, after an injection of bradykinin into the knee-joint. All measurements were made just after peptide injection because of the short-lived nociceptive action of bradykinin.

2.3. Drug injection

Injections of bradykinin, or its B_1 and B_2 receptor antagonists, were made up in 0.9% saline solution and in a volume of 50 μl. Only in the case of co-injections of peptides, or peptides and prostaglandin, did the volume of injections reach 100 μl per articulation. Prostaglandin was stocked up in ethanol solution (500 μg/ml) and freshly diluted in saline (500 ng/50 μl or 1.4 nmol/50 μl) at the moment of injection.

2.4. Drugs

The following compounds were used: bradykinin (molecular weight (MW) 1060.2, Sigma), des-Arg^9-[Leu^8]bradykinin (MW 870, Sigma) a bradykinin B_1 receptor antagonist (Regoli et al., 1977), des-Arg^9-bradykinin (MW 904, Sigma), a bradykinin B_1 receptor agonist (Regoli et al., 1977), HOE-140 (D-Arg^0[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin, MW 1304.6, Hoechst), a bradykinin B_2 receptor antagonist (Hock et al., 1991), indomethacin (Merck, Sharp and Dohme), prostaglandin E_2 (MW 352.5, Sigma).

2.5. Statistical analyses

All statistical analyses were carried out using the INSTAT Graphpad version 2.02 (1990–1993). Multiple comparisons were made using one-way analyses of variance (ANOVA), and when a significance level of at least $P < 0.05$ was detected, analyses were followed by the Bonferroni test. When comparison was made between two means, only the unpaired Student's *t*-test was used. Results are expressed as mean ± S.E.M. of six animals.

3. Results

3.1. Bradykinin incapacitation and its antagonism by co-administration of bradykinin B_1 (des-Arg^9-[Leu^8] bradykinin) and B_2 (HOE-140) receptor antagonists

A single injection of bradykinin (25 nmol) into the knee-joint did not cause incapacitation. However, five consecutive hourly injections induced progressive incapacitation (Fig. 1). Measurements were made immediately after bradykinin injections because the incapacitation response lasted less than 1 min. Repeated injections of saline (50 μl) did not alter normal deambulation (data not shown). Four co-administrations of bradykinin (25 nmol) plus des-Arg^9-[Leu^8]bradykinin (30 nmol) did not cause incapacitation. However, a fifth intra-articular injection of bradykinin caused incapacitation of the same magnitude as in animals

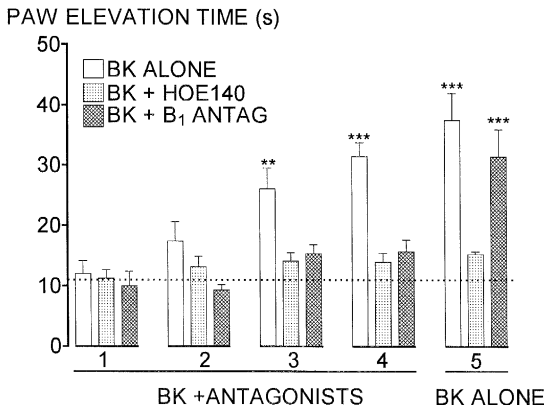

Fig. 1. Incapacitation responses induced by consecutive intra-articular injections of bradykinin, and their blockade by co-administration of bradykinin B_1 (des-Arg^9-[Leu^8]bradykinin) and B_2 (HOE-140) receptor antagonists. The open bars show the duration of the incapacitation measured in the first minute after hourly injections of bradykinin (25 nmol/knee). Numbers 1–5 indicate the sequence of injections. The cross-hatched bars are the incapacitations induced by bradykinin, co-injected with des-Arg^9-[Leu^8]bradykinin (30 nmol/knee). The 5th injection of bradykinin was administered without the antagonist. The stippled bars show the effect of bradykinin co-injected with HOE-140 (0.5 nmol/knee). In the fifth injection, the antagonist was omitted. All bars indicate mean ± S.E.M. paw elevation time of six animals per group. The dotted line represents the mean paw elevation time value of the control group before consecutive injections of bradykinin. **,*** Statistical difference to the control (dotted line) with $P < 0.01$ and $P < 0.001$, respectively.

receiving five successive injections of bradykinin. Four co-injections of bradykinin (25 nmol) and HOE-140 (0.5 nmol) did not cause incapacitation, but in contrast with the experiments with the bradykinin B_1 antagonist, a fifth injection of bradykinin alone was also ineffective in producing incapacitation. The doses of the antagonists used here were chosen among those minor doses that fully inhibited bradykinin-induced incapacitation, in pilot studies.

3.2. Incapacitation induced by the bradykinin receptor agonist des-Arg^9-bradykinin

Five successive hourly injections of des-Arg^9-bradykinin (30 nmol/knee) did not induce incapacitation responses (Fig. 2, left panel). On the other hand, although three consecutive prostaglandin E_2 injections (1.4 nmol/knee) had no effect per se, this treatment sensitized the joints to a previously non-incapacitating dose of the bradykinin B_1 receptor agonist (Fig. 2, right panel). These animals were pretreated with indomethacin (5 mg/kg, i.p.) in order to prevent a possible endogenous release of cyclooxygenase metabolites as a result of the injection trauma.

3.3. Restoration by prostaglandin E_2 of the bradykinin-induced incapacitation inhibited by indomethacin or HOE-140 pretreatment

Pretreating the animals with indomethacin (5 mg/kg, i.p., 60 min before) fully inhibited incapacitation induced by successive injections of bradykinin. However, under the same treatment, the co-administration of bradykinin with

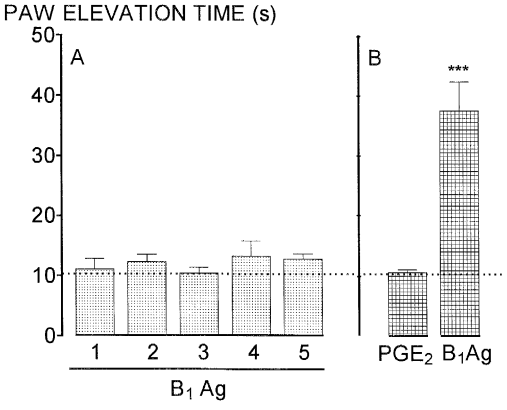

Fig. 2. Effect of successive injections of the bradykinin B_1 receptor agonist des-Arg^9-bradykinin and its potentiation by prostaglandin E_2. Panel A shows that five successive injections of des-Arg^9-bradykinin (30 nmol/knee per h) did not induce incapacitation. Numbers 1–5 indicate the sequence of hourly agonist injections. Panel B shows paw elevation time after three successive prostaglandin E_2 injections (PGE_2,3×1.4 nmol/knee per h). B_1Ag indicates incapacitation induced by the bradykinin B_1 receptor agonist (30 nmol/knee) injected after prostaglandin E_2 treatment. The dotted line represents mean paw elevation time before the injections of des-Arg^9-bradykinin. All bars represent mean ± S.E.M. paw elevation time, $n = 6$. *** Statistical difference between PGE_2 and B_1Ag bar with $P < 0.001$.

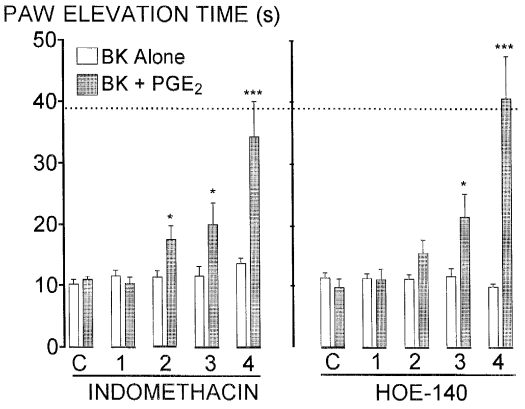

Fig. 3. Prostaglandin E_2 restores bradykinin-induced incapacitation inhibited by indomethacin or HOE-140 pretreatment. The group of bars on the left shows the effect of indomethacin pretreatment (5 mg/kg, i.p., 1 h before) upon bradykinin (4×25 nmol/knee per h)-induced incapacitation (open bars). The stippled bars show the effect of the co-injections with prostaglandin E_2 (1.4 nmol/knee) and bradykinin in animals pretreated with indomethacin. The group of bars on the right shows similar experiments with HOE-140 (5 mg (3.7 μmol)/kg, s.c., 1 h before) instead of indomethacin. Numbers 1–4 indicate the sequence of hourly injections of bradykinin, or bradykinin and prostaglandin E_2. All bars represent mean ± S.E.M. paw elevation time, $n = 6$. The dotted line represents the mean paw elevation time value of the control group (pretreated with saline 0.9%, i.p.) after four consecutive injections of bradykinin. The control bars (C) represent mean paw elevation time values of each group before intra-articular injections, $n = 6$. *,*** Statistical difference to the sided open bar (BK alone) with $P < 0.05$ and $P < 0.001$, respectively.

prostaglandin E_2 (1.4 nmol) restored the incapacitation (Fig. 3, left). Similar results were obtained using HOE-140 (5 mg/kg, s.c., 60 min before) instead of indomethacin (Fig. 3, right). In both cases, the maximal incapacitation observed was equivalent to that of the control group, which was pretreated with saline (1 ml, i.p., 60 min before) and had received four bradykinin intra-articular injections. The same results were obtained for the control group using Tris buffer (vehicle of indomethacin solution), instead of saline (data not shown).

4. Discussion

It is currently accepted that inflammatory nociception is triggered by a noxious or non-noxious activation of a sensitized (hyperalgesic) primary sensory neuron. In algesimetric tests, this activation is detected by means of some characteristic behavioural response. Consequently, we understand 'nociception' as 'the actual expression of behaviour in response to an aggressive stimulus' and 'hyperalgesia' is 'facilitating the occurrence of such behaviour'. Inflammatory algesimetric tests can be broadly divided in two basic types: (i) tests in which the overt behavioural responses are not induced by the inflammatory challenge, but by some exogenous secondary stimuli, as in the rat paw withdrawal test elicited by pressure (Randall and Sellito, 1957; Ferreira et al., 1978) or heating (Hargreaves et al., 1988); or (ii) tests in which, after an inflammatory

stimulus, the animals spontaneously present the behavioural response, as in the abdominal writhing (Siegmund et al., 1957) or paw flinches induced by the formalin test (Dubuisson and Dennis, 1977). Using the former type of test, the inflammatory process will only decrease the latency between the exogenous stimuli and the standard behaviour: thus, we will only observe hyperalgesia. Through the latter type of test, since the behaviour related to nociception is expected to be spontaneous, it is possible to observe when some mediator causes nociception, whether it induces such behaviour, or hyperalgesia, whether it potentiates another agent, or even another injection of itself, to elicit such behaviour. In the test used here, spontaneous flexion of the hindlimb (incapacitation) induced by intra-articular administration of bradykinin was the paradigm for overt nociception.

We have reported here that a single intra-articular administration of bradykinin did not cause nociception. However, additional injections produced progressive incapacitation (Fig. 1), therefore suggesting that successive injections of bradykinin caused sensitization to their own nociceptive effect. We have previously shown, using this model, that articularly administered prostaglandin E_2 did not cause nociception but potentiated the effects of the mediators released by carrageenin (Tonussi and Ferreira, 1992). In the present series of experiments, prostaglandin production seems to be mediating bradykinin-induced sensitization. This suggestion was supported by the fact that consecutive co-injections of bradykinin and prostaglandin E_2 restored incapacitation in animals pretreated with indomethacin, a prostaglandin synthesis inhibitor (Fig. 3). The results confirm a previous report involving the effect of arachidonic acid metabolites on bradykinin-induced hyperalgesia (Taiwo and Levine, 1988).

The data described here also support the idea that bradykinin B_2 receptor is associated with bradykinin sensitization of the knee-joint rather than nociception. HOE-140 pretreatment blocked the nociception by successive injections of bradykinin, but did not affect incapacitation responses to co-administration of bradykinin and prostaglandin E_2. The mechanism involved in the bradykinin B_2 receptor-mediated sensitization was more extensively studied using the rat paw hyperalgesia test and suggests that this bradykinin receptor triggers prostaglandin-releaser cytokines, like tumour necrosis factor-α and interleukin-1β (Ferreira et al., 1993). We cannot discard, however, the possibility of a direct bradykinin B_2 receptor-stimulated prostaglandin production, since it was observed in vitro using fibroblast cultures (Burch and Axelrod, 1987; Conklyn et al., 1988). The observation that HOE-140 did not block the incapacitation induced by co-injection of bradykinin and prostaglandin suggested the possibility that another type of bradykinin receptor was mediating the nociceptive response.

Our results support the participation of bradykinin B_1 receptors in the process of activation of the nociceptors. In fact, successive intra-articular co-injection of bradykinin with bradykinin B_1 receptor antagonist prevented the development of incapacitation. However, 1 h later, a further injection of bradykinin did induce incapacitation, indicating that although the bradykinin B_1 receptor antagonist blocked the nociceptive behaviour, it did not block the development of the knee-joint sensitization. In this situation, a previously non-effective dose of bradykinin is now able to induce nociception (Fig. 1). This idea was further supported by the finding that consecutive injections of the bradykinin B_1 receptor agonist des-Arg^9-bradykinin did not induce incapacitation, but evoked immediate incapacitation when injected into prostaglandin-sensitized joints (Fig. 2). It has been suggested that de novo synthesis and expression of bradykinin B_1 receptors (Marceau et al., 1980; Couture et al., 1982) occur during the inflammatory response. An alternative explanation is that bradykinin B_1 receptors are present constitutively, but the sensitization of the primary sensory neuron by hyperalgesic mediators such as prostaglandin E_2 is crucial to the possibility of activation of the nociceptors by bradykinin. Furthermore, since the classical nociceptor seems to have only bradykinin B_2 receptors (Steranka and Burch, 1991; Farmer and Burch, 1992), it is possible that bradykinin B_1 receptors are still on another type of nociceptive fibres. A possibility is that it would be on the silent nociceptors reported by Schaible and Schmidt (1985).

Recently, using a rat model of articular inflammatory hyperalgesia, it was proposed that bradykinin B_1 receptors also mediate hyperalgesia (Perkins et al., 1993; Davis and Perkins, 1994). However, their experimental approach, which measures the capacity of hindlimbs to sustain an external load applied to the back of animals, does not allow differentiation between sensitization (hyperalgesia) and activation (overt behaviour) of the nociceptors. As explained above, methods which utilize exogenous stimuli do not permit the observation of spontaneous nociception due to inflammation, and animals flexing their knee-joints, due to articular nociception, would also fail to sustain, with the affected hindlimb, the load applied. However, further experiments are necessary in order to clarify this point.

In conclusion, the results presented here indicate that the knee-joint incapacitation test is a useful tool to study, in parallel, the nociceptive and sensitizing action of inflammatory mediators. In this model, the bradykinin-nociceptive response was considered to be mediated by bradykinin B_2 receptor-mediated sensitization and bradykinin B_1 receptor-mediated activation of the nociceptors.

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