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Report

Role of Putative Neurotransmitters in Bradykinin-Induced Catalepsy in the Rat

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Intracerebroventricular administration of bradykinin produced a dose-related cataleptic response in rats. Bradykinin-induced catalepsy was significantly attenuated following pretreatment with pharmacologic agents that decrease central prostaglandin, serotonin, and acetylcholine activity, as well as by the opioid receptor antagonist naloxone. Conversely, pharmacologic treatments that enhance central catecholamine levels and, specifically, central dopaminergic activity also inhibited bradykinin-induced catalepsy. The prostaglandin precursor, arachidonic acid, and prostaglandin E_2, as well as met- and leu-enkephalin showed a synergistic effect with bradykinin catalepsy. Much evidence indicates that several actions of bradykinin, in the central nervous system and the periphery, are likely to be prostaglandin mediated. Further, the recent report from this laboratory that centrally administered bradykinin specifically augments rat brain prostaglandin E_2 levels, together with the proposed role of central prostaglandins as modulators of central synaptic transmission, suggests that bradykinin-induced catalepsy is mediated and modulated through PGE effects on serotonergic, cholinergic, and dopaminergic neurotransmitter systems. The study also indicates that endogenous opioid peptides may be involved in braydkinin catalepsy.

KEY WORDS: bradykinin; catalepsy; prostaglandins; serotonin; acetylcholine; dopamine; enkephalins.

INTRODUCTION

Bradykinin is known to be released in the periphery and has been implicated in a variety of pathological conditions (1). Recent studies have indicated that bradykinin exerts important physiopathological effects on the central nervous system (CNS) and may function as a neuromodulator in the mammalian brain (2). Bradykinin, along with systems capable of synthesizing and inactivating kinins, is present in the CNS of several animal species, including the rat (2,3). Centrally administered bradykinin has been reported to induce catalepsy (2) and to potentiate neuroleptic-induced catalepsy (4) in rats. The mechanism of bradykinin-induced catalepsy is not known but is unlikely to be a direct effect of the autacoid. In its proposed role as a central neuromodulator (2) it appears reasonable to assume that bradykinin-induced catalepsy involves other neurotransmitter systems. This possibility has now been investigated.

MATERIALS AND METHODS

Wistar-strain albino rats (180–220 g) of both sexes were used. The animals were caged individually with free access to standard pellet chow and water, at an ambient temperature of 22 ± 2°C and 45–55% relative humidity, with a 12-hr light–dark cycle. All experiments were conducted at this ambient temperature between 9:00 AM and 2:00 PM.

Catalepsy was quantified by adopting the ring test of Pertwee (5). The rat was placed, after specified drug-pretreatment intervals, on a steel ring, 12 cm in diameter, fixed to a steel stand at a height of 35 cm. The time during which the rat remained motionless, with complete cessation of snout and whisker movements, of the total observation period of 5 min, was used to calculate the "percentage immobility." Naive rats were used each time because tolerance to bradykinin-induced behavioral responses has been observed following repeated administration of the kinin (6).

Graded doses of bradykinin triacetate, dissolved in 10 μl of artificial cerebrospinal fluid (CSF), were administered intracerebroventricularly (icv) to groups of rats through indwelling cannulas inserted stereotaxically into the right lateral ventricle under pentobarbital sodium (40 mg/kg, ip) anesthesia. Earlier studies from this laboratory (7) indicated that bradykinin induced the peak cataleptic response 15 min after icv administration. The catalepsy became progressively less at 30 and 45 min and waned by 60 min. In the present study, bradykinin-induced catalepsy was quantified 15 min after the administration of the kinin, and no attempt was made to assess the time of onset or the duration of the cataleptic response.

The drugs used, with doses, pretreatment times, and routes of administration given in parentheses, to investigate

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the mechanism of bradykinin-induced catalepsy were 5,6-dihydroxytryptamine creatinine sulfate (75 μg/rat, 48 hr, icv), *p*-chlorophenylalanine methyl ester hydrochloride (100 μg/rat, once daily for 3 days, icv), hemicholinium-3 (20 μg/rat, 45 min, icv), atropine sulfate (10 μg/rat, 15 min, icv), diclofenac sodium (50 μg/rat, 1 hr, icv), paracetamol (50 μg/rat, 30 min, icv), hydrocortisone hemisuccinate (25 mg/kg, 30 min, ip), *l*-DOPA (100 mg/kg, 30 min, ip) with benserazide (50 mg/kg, 30 min, ip), with or without pretreatment with diethyldithiocarbamate sodium (300 mg/kg, 3 hr, ip), amantadine hydrochloride (25 mg/kg, 30 min, ip), naloxone hydrochloride (1 mg/kg, 30 min, ip), prostaglandin E_2 (5 μg/rat, 15 min, icv) arachidonic acid (25 μg/rat, 30 min, icv), leu-enkephalin (1 μg/rat, 15 min, icv), and met-enkephalin (1 μg/rat, 15 min, icv).

All the drugs that were administered icv were dissolved in 10 μl of artificial CSF, except prostaglandin E_2 and arachidonic acid, which were suspended in 1% ethanol prior to dilution in 10 μl of artificial CSF. The drugs that were administered ip were dissolved or suspended in normal saline and injected in a volume of 0.5 ml/100 g. The doses mentioned refer to the respective salts. The control animals received equivalent volumes of the vehicle through the appropriate routes. The doses and pretreatment times of the pharmacological agents used were based on data available in this laboratory (8,9), and none of them, apart from prostaglandin E_2, arachidonic acid, and the enkephalins, showed any cataleptic effect per se at the doses used (10,11).

RESULTS

Bradykinin (5, 10, and 20 μg icv) produced a dose-related cataleptic response in rats when assessed 15 min after its administration (Table I). Since bradykinin (5 μg) produced minimal catalepsy, whereas a higher dose (20 μg) produced a submaximal effect, these two doses were used for subsequent studies. Bradykinin catalepsy was preceded by overt signs of central stimulation, characterized by increased spontaneous motility, vocalization, piloerection, and aggressiveness. These effects were more marked with the lower doses (5 and 10 μg) of bradykinin and appeared within 1–3 min and passed off by 8–10 min after the administration of bradykinin.

Bradykinin (20 μg)-induced catalepsy was significantly attenuated after pretreatment with 5,6-dihydroxytryptamine (DHT), which induces selective degeneration of central serotonergic neurons, and *p*-chlorophenylalanine (PCPA), an inhibitor of serotonin biosynthesis, by 50 and 37%, respectively. Similarly, hemicholinium, which inhibits the uptake of choline into the cholinergic neurons and thereby reduces acetylcholine synthesis, and atropine, a cholinergic muscarinic receptor antagonist, inhibited the cataleptic effect of bradykinin by 46 and 32%, respectively. The prostaglandin (PG) synthesis inhibitors, hydrocortisone, which inhibits, the enzyme phospholipase A_2, and the cyclooxygenase inhibitors, diclofenac and paracetamol, markedly reduced the cataleptic effect of bradykinin by 56, 46, and 36%, respectively. The administration of the catecholamine precursor, *l*-DOPA, with benserazide, a peripheral decarboxylase inhibitor, reduced bradykinin-induced catalepsy by 46%. However, when diethyldithiocarbamate (DDC), an inhibitor of dopamine-β-hydroxylase, was added to the regimen so as to ensure selective increase in central dopamine (DA) levels, the catalepsy-inhibiting effect of *l*-DOPA with benserazide increased to 60%. Amantadine, a DA receptor agonist, and naloxone, a specific antagonist of endogenous opiate receptors, attenuated bradykinin-induced catalepsy by 38 and 59%, respectively (Table I).

Further pharmacologic analysis of bradykinin-induced catalepsy was done, based on the results of the aforemen-

Table I. Effects of Some Pharmacologic Agents that Influence Central Transmitter Activity on Bradykinin-Induced Catalepsy in Rats

Groups	*N*	Percentage immobility		*P*[a]
		Mean	SE	
Control (artificial CSF)	10	10.5	3.2	—
Bradykinin (5 μg)	10	24.7	3.9	<0.01[b]
Bradykinin (10 μg)	10	56.4	5.2	<0.001[b]
Bradykinin (20 μg) (BK)	10	79.3	4.6	<0.001[b]
DHT + BK	8	40.2	3.8	<0.001[c]
PCPA + BK	6	49.8	2.9	<0.001[c]
Hemicholinium + BK	6	42.7	3.7	<0.001[c]
Atropine + BK	6	54.2	2.3	<0.001[c]
Hydrocortisone + BK	6	34.6	4.4	<0.001[c]
Diclofenac + BK	5	51.0	3.5	<0.001[c]
Paracetamol + BK	5	42.8	3.9	<0.001[c]
l-DOPA + benserazide + BK	6	42.7	5.7	<0.001[c]
l-DOPA + benserazide + DDC + BK	5	31.8	6.9	<0.001[c]
Amantadine + BK	6	49.3	3.1	<0.001[c]
Naloxone + BK	6	32.5	5.4	<0.001[c]

[a] Student's *t* test used for analysis of the data.
[b] Statistical significance in comparison to the control group.
[c] Statistical significance in comparison to the bradykinin (20 μg) (BK) group.

tioned studies. Arachidonic acid, the PG precursor, and PGE_2 produced a minimal cataleptic effect per se at the doses used in this study. Likewise, the two endogenous opiate peptides, met-enkephalin and leu-enkephalin, induced minimal, although statistically significant, catalepsy at the doses used. All these agents, namely, arachidonic acid, PGE_2, and the enkephalins, augmented the intensity of catalepsy induced by bradykinin (5 μg). This effect appeared to be an additive synergistic phenomenon (Table II).

DISCUSSION

Catalepsy has been defined as a behavioral state in experimental animals, characterized by a poverty of movement and the ability to sustain abnormal postures for a considerable length of time, with a normal righting reflex (12).

A myriad of tests has been devised to quantitate catalepsy in experimental animals. The ring test was chosen for this study because it has been shown to be sensitive and the data obtained are reproducible enough for the assay of cannabinoids (5). Bradykinin, which does not cross the blood–brain barrier, has been reported to induce catalepsy in several animal species following intracerebroventricular administration of the kinin (2). The rabbit has been shown to be the most sensitive species, while the rat is relatively less sensitive to most of the central effects of bradykinin, including catalepsy (2). This may explain the relatively large doses of bradykinin required to induce catalepsy, as was also evident in this study.

Bradykinin-induced catalepsy was preceded by transient but discernible signs of central stimulation. It has been observed that these signs are markedly reduced or even absent when bradykinin is administered icv to rats pretreated with 6-hydroxydopamine (unpublished observations), which is known to induce selective degeneration of central catecholaminergic neurons on icv administration. Bradykinin has been reported to release norepinephrine in a number of tissues (13). It is of interest to note that morphine-induced catalepsy is also preceded by signs of CNS stimulation (11), a feature absent in neuroleptic-induced catalepsy (14).

Bradykinin-induced catalepsy was markedly attenuated following pretreatment of the rats with pharmacologic agents that selectively reduce central serotonergic and cholinergic activity. Conversely, antagonism of the catalepsy was also induced by pharmacologic treatments aimed to enhance central catecholamine levels and, more specifically, brain DA concentrations. The anti-cataleptic effect of amantadine, a DA receptor agonist, provided further evidence for the inhibitory role of DA in bradykinin-induced catalepsy.

The PG synthesis inhibitors, hydrocortisone, diclofenac, and paracetamol, the latter being a selective inhibitor of brain cyclooxygenase (15), markedly antagonized bradykinin-induced catalepsy. On the contrary, the PG precursor, arachidonic acid, and PGE_2 synergized the catalepsy-inducing effect of the kinin. Bradykinin has been reported to enhance PG synthesis by activating phospholipase A_2, thereby increasing the availability of precursors for conversion into PGs (16). Hydrocortisone is known to inhibit the activation of phospholipase A_2 (17), and this may form the basis of the marked anticataleptic effect of the corticoid. The wide spectrum of interactions between bradykinin and PGs has been reviewed (18) and it has been envisaged that PGs function as mediators and modulators of several actions of bradykinin in the periphery. The bradykinin-induced pressor response in rats (19) and hyperthermia in rabbits (20), following central administration of the kinin, have been shown to be PG-mediated effects. PG synthesis inhibitors have been reported to inhibit bradykinin-induced release of PGs and to antagonize many of the central and peripheral actions of bradykinin (2). It has been proposed (21) that bradykinin activates the PG-generating system early in the PG synthesis cascade by stimulating phospholipase A_2 (16,21). Recent studies from this laboratory (22) have shown that catalepsy-inducing doses of bradykinin produce a significant and selective increase in rat brain PGE_2 concentrations but

Table II. Effects of Arachidonic Acid, PGE_2, and Enkephalins on Bradykinin-Induced Catalepsy in Rats

Groups	*N*	Percentage immobility		*P*[a]
		Mean	SE	
Vehicle (1% ethanol in artificial CSF)	6	16.2	3.4	—
Arachidonic acid	6	29.3	2.9	<0.05[b]
PGE_2	6	24.9	2.2	<0.05[b]
Vehicle (artificial CSF)	6	9.8	3.0	—
Met-enkephalin	5	31.5	3.6	<0.01[b]
Leu-enkephalin	5	36.2	3.9	<0.01[b]
Bradykinin (5 μg) (bk)	10	24.7	3.9	<0.01[b]
Arachidonic acid + bk	6	66.4	6.8	<0.001[c]
PGE_2 + bk	6	59.6	5.9	<0.001[c]
Met-enkephalin + bk	6	55.9	6.6	<0.001[c]
Leu-enkephalin + bk	6	61.6	5.6	<0.001[c]

[a] Student's *t* test used for analysis of the data.
[b] Statistical significance in comparison to the respective vehicle-treated group.
[c] Statistical significance in relation to the bradykinin (5 μg) (bk) group or the respective drug-treatment group.

not that of $PGF_2\alpha$ levels. The maximal increase in rat brain PGE_2 levels was noted 15 min after icv administration of bradykinin, and the augmented PGE_2 concentrations declined to near-normal levels by 60 min, correlating well with the time course of catalepsy induced by the kinin. Bradykinin has been reported earlier to induce specifically the release of PGEs in many peripheral tissues (18).

In view of the considerable evidence indicating that bradykinin actions are likely to be PG mediated and that PGs function as modulators in the mammalian CNS (23), it appears possible that the observed involvement of serotonin, acetylcholine, and DA in bradykinin-induced catalepsy might be a consequence of PG-induced modulation of these neurotransmitter activities. A modulatory role for PGs in rat brain serotonergic activity has been envisaged and the data have been reviewed recently (24). PGE_1 has been shown to enhance the turnover of serotonin in the rat brain, whereas $PGF_2\alpha$ was reported to decrease it (25,26). Bradykinin-induced increase in rat brain serotonin concentrations were reported to be antagonized following pretreatment with the PG synthesis inhibitors, hydrocortisone, diclofenac, and paracetamol (22), suggesting that the bradykinin-induced increase in rat brain PGE_2 levels may be responsible for the augmented serotonergic activity. It was noted that the pattern of increase in the rat brain PGE_2 and serotonin concentrations, induced by bradykinin, followed a similar time course (22).

The other neurotransmitters whose activities are believed to be modulated by PGs are acetylcholine and catecholamines. PGs of the E series have been shown to enhance rat brain acetylcholine concentrations (27) and to inhibit the release of DA from rat striatum (28). These observations are in accord with the postulate that bradykinin induces catalepsy through a PGE-induced mechanism, since pharmacologic agents that attenuate central cholinergic activity or augment central dopaminergic activity inhibited the catalepsy. In a recent communication (29), centrally administered PGE_1 has been shown to induce catalepsy in rats, which, like bradykinin-induced catalepsy, was attenuated by pharmacologic treatments known to reduce central serotonergic and cholinergic activity and to induce a selective increase in brain DA levels.

Bradykinin has been reported to induce a rise in both cyclic AMP and cyclic GMP levels and to alter the permeability of the neuronal membrane to calcium ions (13,30). PG synthesis inhibitors prevented the rise in cyclic AMP induced by bradykinin but did not affect the rise in cyclic GMP, suggesting that the kinin effect on cyclic AMP is a consequence of enhanced PG synthesis and release (30). There is a possibility that the effects of bradykinin on serotonin, acetylcholine, and dopamine, resulting in catalepsy, are a consequence of the action of the kinin on cyclic AMP or the induced increase in intracellular calcium concentrations in the neurons. However, the data are insufficient to reach any valid conclusion.

The antagonism of bradykinin-induced catalepsy by naloxone and the synergistic effect afforded by met- and leu-enkephalin suggest that endogenous opiate peptides may be involved in the kinin action. Morphine has been reported to potentiate bradykinin catalepsy in rabbits (31). The analgesic activity of centrally administered bradykinin and its congeners has been shown to be antagonized by naloxone pretreatment (32). It was suggested that bradykinin acted directly on a kinin-specific receptor, which led to activation of the opiate receptor (32).

It is not known whether an interrelationship exists between PGs and endogenous opiate peptides. Naloxone has been reported to inhibit the increase in rat brain PGs induced by met-enkephalin (33). However, apart from this isolated report, the literature is silent on this aspect. It is interesting to note that PGE_1-induced catalepsy (29) and the antinociceptive effect of centrally administered PGE_1 (34) and PGD_2 (35) were antagonized by naloxone.

In conclusion, the present study, in conjunction with the earlier reported biochemical data indicating that centrally administered bradykinin specifically augments rat brain PGE_2 levels (22), suggests that bradykinin-induced catalepsy is a consequence of the PGE-modulated increase in rat brain serotonin and acetylcholine activity and decrease in dopaminergic activity. Endogenous opioid peptides also appear to be involved but an interrelationship between the bradykinin-induced increase in rat brain PG activity and these peptides must await future elucidation.

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