0026-895X/90/060785-05$02.00/0

MOLECULAR PHARMACOLOGY, 37:785–789



*ACCELERATED COMMUNICATION*

# Functional Expression of $B_2$ Bradykinin Receptors from Balb/c Cell mRNA in *Xenopus* Oocytes

LAWRENCE C. MAHAN and RONALD M. BURCH

*Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, Maryland 20892 (L.C.M.), and Nova Pharmaceutical Corporation, Baltimore, Maryland 21224 (R.M.B.)*

Received December 26, 1989; Accepted March 19, 1990

**SUMMARY**

The murine BALB/c 3T3 fibroblast clone SV-T2 (3T3 cells) expresses receptors for the nonapeptide bradykinin. In these cells, bradykinin stimulates both inositol phosphate (InsP) formation and arachidonic acid release by independently activating phospholipase C and phospholipase $A_2$, respectively. These actions of bradykinin are mediated by a receptor(s) coupled to pertussis toxin-insensitive guanine nucleotide-binding proteins. Bradykinin-stimulated increases in InsP lead to the mobilization of intracellular $Ca^{2+}$. We examined the expression of 3T3 receptors for bradykinin in oocytes from *Xenopus laevis*, cells capable of *in vitro* expression of foreign mRNA for receptors coupled to the mobilization of $Ca^{2+}$. Poly(A)$^+$ mRNA was prepared from 3T3 cells and expression of receptors for bradykinin was demonstrated by agonist-mediated stimulation of $^{45}Ca^{2+}$ efflux from oocytes injected with 50 ng of poly(A)$^+$ RNA. Bradykinin-stimulated efflux of $^{45}Ca^{2+}$ was dose dependent ($EC_{50}$ = 15 nM) and blocked by the specific mixed $B_1$,$B_2$ bradykinin antagonist NPC 567 but not by the $B_1$ antagonist desArg$^9$[Leu$^8$]bradykinin. Size fractionation of 3T3 poly(A)$^+$ RNA on a sucrose gradient demonstrated a single peak of bradykinin-stimulated $^{45}Ca^{2+}$ efflux, with an approximate mRNA size of 4.5 kilobases. Bradykinin-stimulated $^{45}Ca^{2+}$ efflux in size-fractionated mRNA was clearly separable from response to [Arg]vasopressin at another receptor linked to InsP formation and $Ca^{2+}$ mobilization in 3T3 cells.

Receptors for the nonapeptide bradykinin are widely distributed throughout mammalian tissues and have been shown to mediate a number of specific responses including neurotransmitter release, vasodilation, smooth muscle contraction, fluid secretion, and pain (1–3). Two subtypes of bradykinin receptors, termed $B_1$ and $B_2$, have been postulated, based on the pharmacological discrimination of synthetic bradykinin analogs in smooth muscle contraction assays (1). More recent studies, however, have shown that this simple classification of subtypes of bradykinin receptors cannot fully account for the properties of these receptors on cells from a variety of tissues (3). In some cells, bradykinin has been shown to cause increases in InsP formation through the activation of phosphatidylinositol-specific phospholipase C and subsequent mobilization of intracellular $Ca^{2+}$. In other cells, bradykinin appears to directly activate phospholipase $A_2$, resulting in the release of arachidonate and subsequent synthesis of prostaglandin $E_2$. Both of these bradykinin-stimulated responses exist in Swiss albino mouse 3T3 cells and BALB/c (SV-T2) mouse 3T3 cells and have been shown to involve receptors coupled to pertussis toxin-insensitive G proteins (4, 5). Pharmacologically, however, receptors for bradykinin on 3T3 cells belong to the $B_2$ subtype (6).

[1] Unpublished observations.

In the past few years, cDNAs for a number of receptors of the G protein-coupled superfamily have been cloned. In particular, the demonstration of multiple transduction pathways and molecular heterogeneity within the muscarinic (7), adrenergic (8), and serotonergic classes of receptors (9) has emphasized the inadequacy of classic pharmacological subtyping of receptors. In general, fewer pharmacological subtypes have been proposed for peptide receptors; however, the existence of multiple G protein-coupled transduction mechanisms for any single peptide receptor suggests that a similar molecular heterogeneity may exist. In an initial attempt to define the molecular basis of the heterogeneity of receptors for bradykinin, we have expressed mRNA for these receptors from 3T3 cells in oocytes from *Xenopus laevis*.

## Materials and Methods

**Cell culture.** The murine BALB/c 3T3 fibroblast line SV-T2 was obtained from the American Type Culture Collection (ATCC CCL 163.1) and grown in Dulbecco's modified Eagle's medium containing 4.5 g/liter glucose, 10% calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.2 μg/ml fungizone (GIBCO). Cells were maintained at 37° in 10% $CO_2$ in 10-cm tissue culture dishes.

**Preparation of mRNA.** Total RNA was isolated from 100 dishes

**ABBREVIATIONS:** InsP, inositol phosphate; G protein, GTP-binding protein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MOPS, 3-(*N*-morpholino)propanesulfonic acid; MBS, modified Barth's solution; Kb, Kilobase; $InsP_3$, inositol triphosphate.

of 3T3 cells, as previously described (10). When cells were 80% confluent, they were washed once with 10 ml of phosphate-buffered saline, pH 7.4, and lysed directly in the dishes in a buffer containing 5.5 M guanidine thiocyanate, 25 mM sodium citrate, 0.5% sodium lauryl sarcosine, and 0.2 M 2-mercaptoethanol, pH 7.0 (GNTC solution). DNA was sheared by three passes each through a 19-gauge and a 23-gauge needle, and the final volume was adjusted to 240 ml. The GNTC lysate was divided (~20 ml) and layered directly onto 17 ml of solution containing cesium trifluoroacetate and 0.1 M EDTA, pH 7.0, at a final density of 1.51 g/ml. RNA was separated from DNA and cellular proteins at 25,000 rpm in SW28 rotors for 23–24 hr at 15°. RNA pellets were dissolved in 0.4 ml of 4 M GNTC solution, and total RNA was precipitated in acetic acid and ethanol. After a second NaCl/ethanol precipitation, poly(A)$^+$ mRNA was separated from total RNA by two rounds of oligo(dT)-cellulose chromatography (11).

Size fractionation of mRNA was carried out as previously described (12). mRNA (190 μg in sterile water) was heated to 65° for 5 min before sedimentation through a 10–30% sucrose gradient containing 10 mM HEPES, pH 7.5, 1 mM EDTA, and 0.1% lithium dodecyl sulfate. Samples were centrifuged at 4° for 18 hr in a Beckman SW41 rotor at 40,000 rpm (200,000 × *g*). Fractions (0.33 ml each) were collected and maintained as 50% ethanol solutions at −80°. RNA was quantified using a Beckman DU65 UV spectrophotometer ($A_{260}$ unit = 40 μg/ml). Sucrose gradient analysis was performed by measurement of refractive index. The size distribution of mRNA in pooled density gradient fractions was determined by denaturing agarose gel electrophoresis (13). Approximately 75–100 ng of pooled mRNA from three fractions were dried, resuspended in tracking dye containing 50% formamide, and denatured at 95° for 2 min. Samples were immediately electrophoresed in 1% agarose (SeaKem GTG; FMC BioProducts) containing 20 mM MOPS, pH 6.0, 5 mM sodium acetate, 1 mM EDTA, and 0.66 M formaldehyde. RNA ladder markers, 0.24–9.5 kb (BRL), were visualized by staining with 2 μg/ml ethidium bromide in sterile distilled water. Gradient fractions and markers were transferred electrophoretically onto Genescreen nitrocellulose paper (NEN), according to the manufacturer's instructions. Fractions containing sized mRNA were visualized by autoradiography after hybridization with a poly(dT)$_{20}$ oligomer that had been tailed with [$^{32}$P]TTP (NEN), using terminal deoxynucleotide transferase (Boehringer Mannheim), to a specific activity of ~5.7 × 10$^7$ dpm/μg (14).

**Oocyte injection and culture.** Mature oocyte-positive female *X. laevis* were obtained from Nasco (Fort Atkinson, WI) and maintained in aquatic culture. Animals were anesthetized by immersion in ice-cold water for 15–20 min and ovarian lobes were removed by abdominal incision. The largest oocytes were isolated by manual dissection and maintained in MBS containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 15 mM HEPES, 0.7 mM $CaCl_2$, and 0.8 mM $MgSO_4$ supplemented with 5 mM sodium pyruvate, 10 μg/ml penicillin, 10 μg/ml streptomycin, and 0.2 μg/ml fungizone (15). After 18–24 hr at 18°, viable oocytes were injected in the vegetal pole with either 50 nl of sterile water or mRNA prepared from NaCl/ethanol precipitates from individual (1.5 μg) or pooled fractions (3 × 500 ng) and resuspended in sterile water to a final concentration of 1 μg/μl. Oocytes were stored in MBS, which was changed daily until oocytes were assayed for $^{45}Ca^{2+}$ efflux at the time indicated.

**$^{45}Ca^{2+}$ efflux assay.** The determination of $^{45}Ca^{2+}$ efflux from injected oocytes was performed, with minor modifications, as previously described (16). Oocytes containing follicle cells were incubated in MBS containing 20 μCi/ml $^{45}Ca^{2+}$ (41 Ci/g; NEN) for 18–24 hr. After washing three times with MBS, groups of four oocytes were placed into 200 μl of MBS in the wells of 96-well flat-bottomed plates. Using this procedure, eight sets of oocytes could be simultaneously processed using an eight-channel adjustable micropipette. Media (180 μl) were collected and replaced every 10 min for 50 min and then every 5 min thereafter. (In early experiments, 100 μl were replaced at each time point, but this was found to dilute the signal to an unacceptable level.) Bradykinin, 1 μM or as indicated, was added at the 80-, 85-, and 90-min times. Basal (control) release was defined as the mean release in the 65-, 70-, and 75-min aliquots. Response to carbachol, 100 μM or 1 mM, was measured at 110 min to assess viability of oocytes that did not respond to bradykinin. Using this protocol, control $^{45}Ca^{2+}$ efflux ranged from 200 to 650 dpm in the various experiments. Radioactivity in each collected aliquot was measured by liquid scintillation counting.

**InsP formation.** Quantitation of InsP formation was performed essentially as described (17). Oocytes were labeled 18–24 hr with 50 μCi/ml *myo*-[$^3$H]inositol. The oocytes were then washed three times and incubated in MBS containing 10 mM LiCl for 15 min, after which 1 μM bradykinin was added for 15 min. Incubations were terminated by the addition of perchloric acid to 5% (w/v) and the disruption of the oocytes with a pipette tip while incubating on dry ice. The solution was extracted with 1:1 trichlorotrifluoroethane/tri-*n*-octylamine, and InsPs were separated by high pressure liquid chromatography on a strong anion exchange column (Beckman), using a linear gradient of aqueous ammonium phosphate, pH 3.35, 0–0.6 M over 20 min. Radioactivity was detected with an on-line liquid scintillation monitor (Radiomatic).

## Results

**Bradykinin-stimulated $^{45}Ca^{2+}$ efflux in oocytes.** When oocytes injected with 3T3 cell mRNA were $^{45}Ca^{2+}$ loaded, washed, and incubated with 1 μM bradykinin, a rapid efflux of $^{45}Ca^{2+}$ was observed (Fig. 1). This rapid phase of efflux (5–10 min) was followed by a more prolonged phase of efflux lasting up to 20 min. Water-injected oocytes maintained a constant basal efflux during this time that was unaffected by the addition of bradykinin (data not shown). Both mRNA-injected and control oocytes responded to the addition of 100 μM to 1 mM carbachol, indicating that endogenous muscarinic-stimulated $Ca^{2+}$ mobilization was present. Bradykinin stimulated $^{45}Ca^{2+}$ efflux in injected oocytes approximately 2–3-fold over basal efflux. The magnitude of bradykinin-stimulated efflux was proportional to the amount of mRNA injected (data not shown).

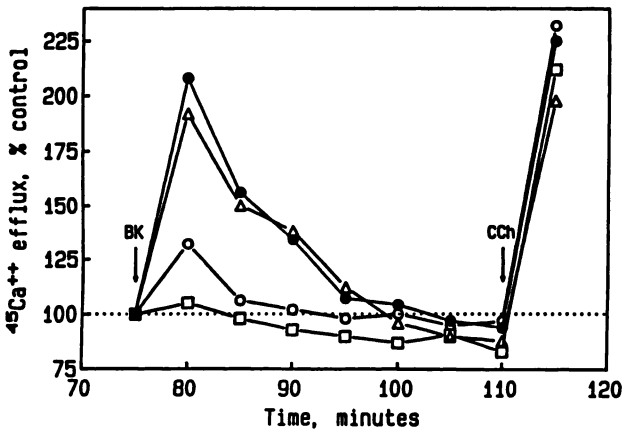


**Fig. 1.** Bradykinin stimulates $^{45}Ca^{2+}$ efflux from *Xenopus* oocytes injected with 3T3 mRNA. Groups of four oocytes, injected with 50 ng of mRNA, were incubated for 3 days in MBS, followed by an additional 12–18 hr in the presence of $^{45}Ca^{2+}$. Oocytes were washed in MBS without $^{45}Ca^{2+}$ and periodic changes of MBS were made until basal $^{45}Ca^{2+}$ efflux did not change (60 min). At 75 min, the indicated concentrations of bradykinin (*BK*) and/or bradykinin analogs were added and $^{45}Ca^{2+}$ efflux was measured in supernatants collected at 5-min intervals. ●, bradykinin (1 μM); △, bradykinin plus desArg$^9$[Leu$^8$]bradykinin (1 μM each); ○, bradykinin (1 μM) plus NPC 567 (10 μM); □, desArg$^9$bradykinin (1 μM). At 110 min, carbachol (100 μM; *CCh*) was added for 5 min and $^{45}Ca^{2+}$ efflux was measured to test for endogenous responsiveness. Data are from a single experiment, representative of results obtained in four experiments using three different mRNA preparations.

Bradykinin-induced efflux was maximum on day 3 after mRNA injection and rapidly deteriorated at later times (data not shown). Thus, all data presented in this report were obtained on day 3 after injection.

**Properties of bradykinin receptors in oocytes.** Bradykinin receptors expressed in oocytes demonstrated properties similar to those of receptors on 3T3 cells (Fig. 1). In contrast to the stimulation of $^{45}Ca^{2+}$ efflux produced by 1 μM bradykinin, the $B_1$-specific agonist desArg$^9$ bradykinin at 1 μM did not elicit $^{45}Ca^{2+}$ efflux. Similarly, the $B_1$-specific antagonist des-Arg$^9$[Leu$^8$]bradykinin at 1 μM did not block bradykinin-induced $^{45}Ca^{2+}$ efflux. Moreover, the $B_1$,$B_2$ antagonist NPC 567 at 10 μM nearly completely blocked bradykinin-induced $^{45}Ca^{2+}$ efflux. Addition of bradykinin over a concentration range of 0.1 nM to 10 μM caused a dose-dependent increase in the magnitude of the $^{45}Ca^{2+}$ efflux (Fig. 2). The concentration of bradykinin that elicited half-maximal response ($EC_{50}$) was 15 ± 1.1 nM ($n$ = 3). This concentration was somewhat greater than the $EC_{50}$ (0.5 nM) in 3T3 cells (4) and may have resulted from metabolism of bradykinin by the follicular cells. This possibility was investigated by incubation of oocytes with bradykinin, followed by analysis of the supernatant for degradation using high pressure liquid chromatography (18). Approximately 85% of 10 nM bradykinin was recovered intact after a 5-min incubation with oocytes (data not shown). In addition, inclusion of 10 μM captopril did not alter the magnitude of $^{45}Ca^{2+}$ efflux elicited by bradykinin (data not shown). Thus, in the absence of significant degradation, this difference could reflect a potential difficulty of bradykinin in traversing the follicle cells surrounding the oocytes or, perhaps, differences in agonist affinity due to coupling of the expressed bradykinin receptor to endogenous G protein(s) in the oocyte. $^{45}Ca^{2+}$ efflux experiments were usually performed with the follicle cell layer intact, to preserve the integrity of the oocytes during the many washes required for the experiment. However, we observed bradykinin-stimulated efflux whether or not the follicle cell layer was present.

Several calcium-mobilizing receptors expressed in oocytes also increase InsP turnover in these cells (17, 19). When 3T3 mRNA-injected oocytes were prelabeled with [$^3$H]inositol and then exposed to 1 μM bradykinin for 15 min, an increase in [$^3$H]Inositol-1,4,5-trisphosphate was detected (Fig. 3). This response was similarly blocked by NPC 567 (data not shown).

**Size fractionation of 3T3 mRNA.** Poly(A)$^+$ mRNA from 3T3 cells was subjected to sucrose density gradient (10–30%) centrifugation to determine the size of the mRNA that encodes functional receptors for bradykinin from 3T3 cells. RNA distributed over the entire gradient, with little aggregated material present in the last fraction (Fig. 4, *top*). Starting with fraction 3, 500 ng from every three fractions were pooled, precipitated by NaCl/ethanol, dried, and resuspended at 1 μg/μl in sterile distilled water for injection. Groups of oocytes (four each) were injected with 50 nl each from the pooled fractions and assayed for increases in $^{45}Ca^{2+}$ efflux elicited by 1 μM bradykinin (Fig. 4, *bottom*). A peak activity of bradykinin-stimulated $^{45}Ca^{2+}$ efflux was observed in pooled gradient fractions 7–9, corresponding to an average mRNA size range of 4–5 kb. This peak of bradykinin-stimulated $^{45}Ca^{2+}$ efflux observed in the gradient fractions was clearly separable from increases in $^{45}Ca^{2+}$ efflux

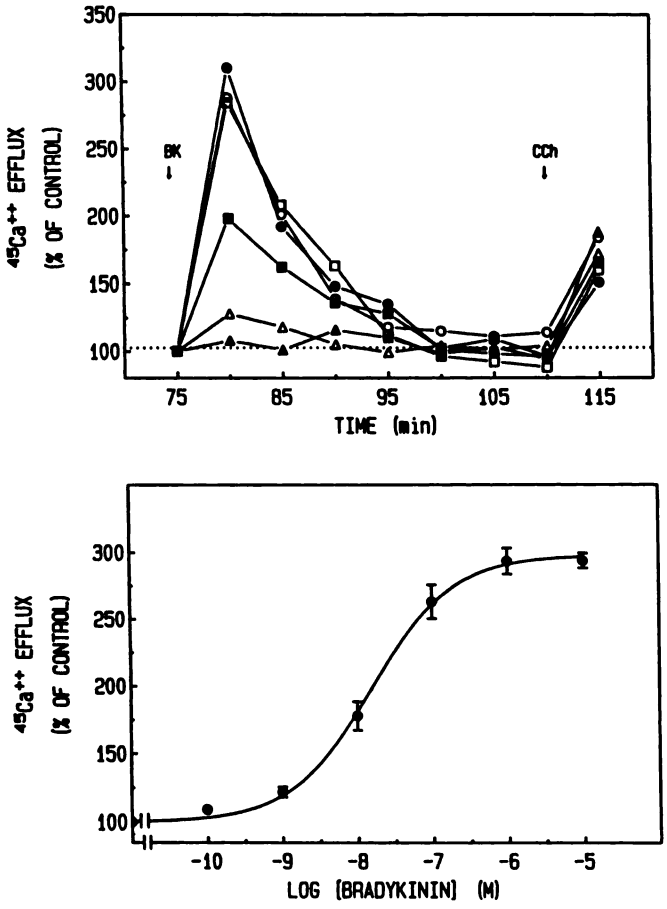


**Fig. 2.** Bradykinin stimulation of $^{45}Ca^{2+}$ efflux in injected oocytes is concentration dependent. *Top*, oocytes, injected and incubated as described in Fig. 1, were stimulated at 75 min with the following concentrations of bradykinin (*BK*) 0.1 nM (▲), 1.0 nM (△), 10 nM (■), 100 nM (□), 1 μM (●), and 10 μM (○). Supernatants were collected at 5-min intervals and $^{45}Ca^{2+}$ efflux was measured. Response to carbachol (100 μM; *CCh*) was determined at 110 min. *Bottom*, data from three experiments were converted into a dose-response curve. Peak responses to different concentrations of bradykinin were analyzed by nonlinear least-squares regression analysis (Marquardt algorithm). The calculated $EC_{50}$ for stimulation of $^{45}Ca^{2+}$ efflux by bradykinin was 15 ± 1.1 nM.

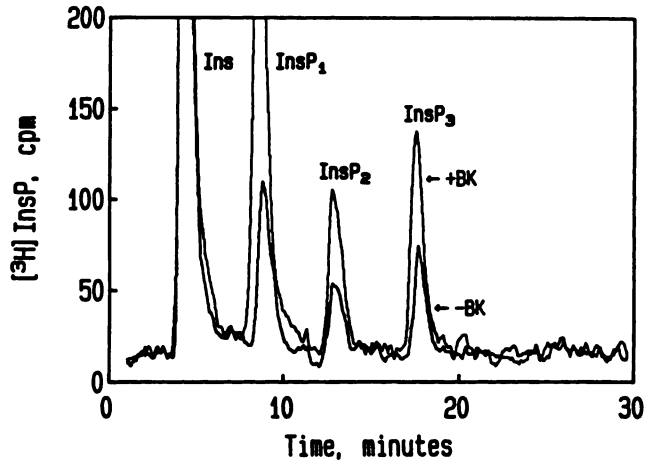


**Fig. 3.** Bradykinin stimulates InsP formation in *Xenopus* oocytes injected with 3T3 mRNA. Oocytes were injected and incubated as in Fig. 1, except that the final 12–18-hr incubation was carried out in the presence of 50 μCi/ml *myo*-[$^3$H]inositol. Oocytes were washed and then bradykinin (1 μM; *BK*) was added. After 15 min, [$^3$H]InsPs were extracted and separated by strong anion exchange high pressure liquid chromatography. Quantitation was performed using an on-line flow-through scintillation detector. ($InsP_1$, inositol monophosphate; $InsP_2$, inositol bisphosphate.

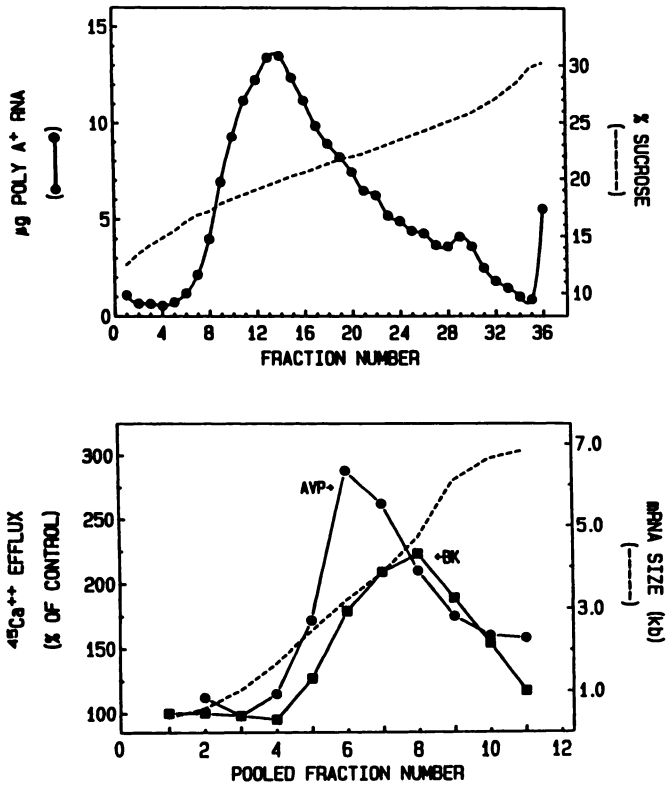


**Fig. 4.** Size fractionation of 3T3 cell mRNA produces a single peak of bradykinin-stimulated $^{45}Ca^{2+}$ efflux activity in oocytes. *Top*, Poly(A)$^+$-selected RNA (190 μg) was fractionated by size over a 10–30% sucrose gradient at 200,000 × *g* for 18 hr at 4°. Fractions of 0.33 ml were collected and assayed spectrophotometrically for RNA content (●). An identical balance gradient was assayed for percentage of sucrose by measurement of refractive index (– – –). *Bottom*, beginning with fraction 3, 500 ng from every three fractions were pooled, precipitated, and resuspended in 1 μl of sterile distilled water for oocyte injection. Pool fraction 12, representing only gradient fraction 36, contained aggregated mRNA and was toxic to the oocytes. Groups of injected oocytes (four) were assayed for stimulation of $^{45}Ca^{2+}$ efflux by 1 μM bradykinin (■) or 1 μM [Arg]vasopressin (●), as described. Aliquots (75–100 ng) of pooled fractions of 3T3 mRNA were electrophoresed under denaturing conditions, transferred to nitrocellulose, and visualized by autoradiography after hybridization with a [$^{32}$P]TTP-tailed oligo(dT)$_{20}$ probe. Size determination of pooled fractions (– – –) was calculated relative to a 0.24–9.5 kb RNA ladder.

elicited by 1 μM [Arg]-vasopressin at another receptor coupled to InsP formation and $Ca^{2+}$ mobilization in 3T3 cells. Subsequent injection of oocytes with mRNA from pool 8 demonstrated the specificity of this bradykinin-stimulated $^{45}Ca^{2+}$ efflux (Fig. 5). The increase in $^{45}Ca^{2+}$ efflux produced by 1 μM bradykinin was markedly attenuated in the presence of 10 μM NPC 567.

## Discussion

Bradykinin and the receptors that mediate its actions play a prominent role in the body's response to trauma and injury (1, 2). Cells in diverse tissues express bradykinin receptors, such as fibroblasts (4), vascular endothelium (6), smooth muscle (20), adipocytes (21), pituitary lactotrophs (22), secretory epithelium (23), glomeruli (24), and kidney tubules (25). Appropriately, therefore, affects of bradykinin have been demonstrated in blood pressure regulation, gut motility, parturition, growth factor release from fibroblasts and white blood cells,

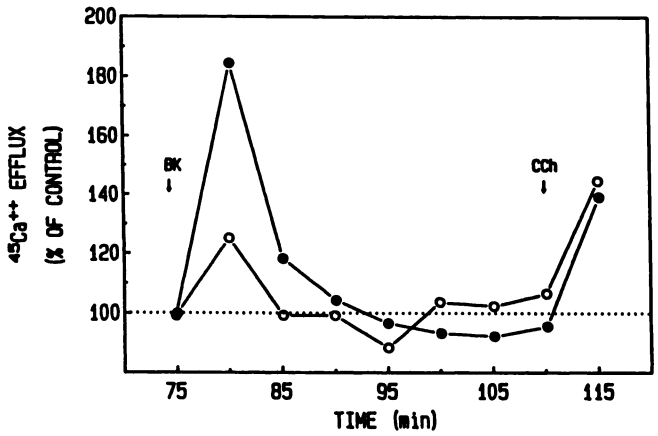


**Fig. 5.** The specific bradykinin antagonist NPC 567 blocks bradykinin-stimulated $^{45}Ca^{2+}$ efflux from oocytes injected with size-selected 3T3 cell mRNA. Oocytes were injected with 50 ng each from peak pooled fraction 8 (Fig. 4, *bottom*) and incubated as described in Fig. 1. $^{45}Ca^{2+}$ efflux was measured in the presence of 1 μM bradykinin (●; *BK*) or 1 μM bradykinin plus 10 μM NPC 567 (○). Response to 100 μM carbachol (*CCh*) was determined at 110 min.

vascular permeability, and lipid metabolism. The presence of bradykinin in nervous tissue and of receptors for bradykinin in the sensory fibers of nociceptive pathways suggests a central role as a neurotransmitter as well (26, 27). To date, receptors for bradykinin have been divided into two subtypes, $B_1$ and $B_2$, based on the pharmacological discrimination of analogs of bradykinin for a variety of physiological responses (1–3). More recently a third subtype, $B_3$, has been proposed (28). Studies conducted with cultured cell models have elucidated at least two mechanisms of transduction for bradykinin. In particular, murine 3T3 cells express $B_2$-type receptors for bradykinin that stimulate both InsP formation and arachidonic acid release by the independent activations of phospholipase C and phospholipase $A_2$, respectively (4).

$B_2$ bradykinin receptors most certainly belong to the G protein-coupled superfamily of receptors. In some cells, such as the neuroblastoma × glioma NG108-15 hybrid (29) and the neuroblastoma × dorsal root ganglion F-11 hybrid (30), bradykinin-stimulated activation of phospholipase C is blocked by the addition of pertussis toxin. Receptors for bradykinin on 3T3 cells stimulate membrane GTPase activity and couple to the activation of phospholipase C and phospholipase $A_2$ by pertussis toxin-insensitive G proteins (4, 5).

Oocytes from *X. laevis* are well suited for the expression of foreign mRNA for receptors that couple to G proteins that activate phospholipase C (16, 17, 19). *Xenopus* oocytes with follicle cells contain muscarinic receptors that, when occupied by agonists, couple to G proteins (either $G_i$ or $G_o$) that are present in mature oocytes (31) and activate phosphatidylinositol-specific phospholipase C. Endogenous muscarinic receptors or expressed receptors that activate phosphatidylinositol-specific phospholipase C cause increases in InsP, in particular $InsP_3$, which mediates the release of $Ca^{2+}$ from intracellular stores. Intracellular mobilization of $Ca^{2+}$ in the oocytes leads to the activation of $Ca^{2+}$-dependent $Cl^-$ channels (19). Thus, expression of receptors may be followed by measurements of $^{45}Ca^{2+}$ efflux or $Cl^-$ ion conductance.

Using *Xenopus* oocytes injected with mRNA from 3T3 cells, we have expressed receptors for bradykinin that stimulate activation of phospholipase C, formation of $InsP_3$, and release

of intracellular $Ca^{2+}$. Stimulation of uninjected oocytes with bradykinin did not lead to formation of InsP or release of $Ca^{2+}$. In this study, we chose to use the SV-T2 3T3 cell line. This line is derived from BALB/c mice and is pharmacologically identical to Swiss albino mouse-derived 3T3 cells in its responses to bradykinin (32). However, SV-T2 3T3 cells express about 3000 binding sites/cell (5), compared with 500 in Swiss 3T3 cells.[1] In these cells, bradykinin and other nonselective agonists stimulate $InsP_3$ and prostaglandin synthesis, whereas the specific $B_1$ agonist desArg$^9$bradykinin does not. Furthermore, mixed $B_1$, $B_2$ antagonists such as NPC 567 block bradykinin-induced effects, whereas the specific $B_1$ antagonist desArg$^9$[Leu$^8$]bradykinin does not. Identical pharmacology was observed for bradykinin-stimulated $^{45}Ca^{2+}$ efflux in oocytes injected with 3T3 cell RNA. Thus, we have functionally expressed murine $B_2$ bradykinin receptors in the oocytes.

When mRNA derived from 3T3 cells was fractionated on a sucrose gradient, a single peak of specific bradykinin-stimulated $^{45}Ca^{2+}$ efflux was observed. The peak fraction of mRNA containing this activity was approximately 4–5 kb in size. These data suggest that the receptor for bradykinin coupled to activation of phospholipase C in 3T3 cells is encoded by a single mRNA species. Sized mRNA encoding bradykinin receptors was clearly separated from mRNA species. Sized mRNA encoding bradykinin receptors was clearly separated from mRNA (approximately 3–4 kb) for [Arg]vasopressin receptors on 3T3 cells, which stimulate $^{45}Ca^{2+}$ efflux in oocytes as well. Whether a separate species of mRNA encoding receptors for bradykinin that couple to the activation of phospholipase $A_2$ is present in these cells is currently under investigation. One intriguing possibility is that 3T3 cells contain two distinct receptors for bradykinin, each coupled to a different G protein that activates either phospholipase C or phospholipase $A_2$. Elucidation of potential molecular heterogeneity of receptors for bradykinin in these cells will help resolve previously published observations on the discrepancies in their pharmacological classification (6).

**References**


1. Regoli, D., and J. Barabe. Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.* **32**:1–46 (1980).
2. Miller, R. J. Bradykinin highlights the role of phospholipid metabolism in the control of nerve excitability. *Trends Neurosci.* **10**:226–228 (1987).
3. Burch, R. M., S. G. Farmer, and L. R. Steranka. Bradykinin receptor antagonists. *Med. Res. Rev.*, **10**:237–269 (1990).
4. Burch, R. M., and J. Axelrod. Dissociation of bradykinin-stimulated arachidonic acid release from inositol phosphate formation in Swiss 3T3 fibroblasts: evidence for a G protein-coupled phospholipase $A_2$. *Proc. Natl. Acad. Sci. USA* **84**:6374–6378 (1987).
5. Burch, R. M., J. R. Connor, and J. Axelrod. Interleukin 1 amplifies receptor-mediated activation of phospholipase $A_2$ in 3T3 fibroblasts. *Proc. Natl. Acad. Sci. USA* **85**:6306–6309 (1988).
6. Conklin, B. R., R. M. Burch, L. R. Steranka, and J. Axelrod. Distinct bradykinin receptors mediate stimulation of prostaglandin synthesis by endothelial cells and fibroblasts. *J. Pharmacol. Exp. Ther.* **244**: 646–649 (1988).
7. Bonner, T. I. The molecular basis of muscarinic receptor diversity. *Trends Neurosci.* **12**:148–151 (1989).
8. Lefkowitz, R. J., and M. G. Caron. Adrenergic receptors. *Adv. Second Messenger Phosphoprotein Res.* **21**:1–10 (1988).
9. Hartig, P. R. Molecular biology of 5-HT receptors. *Trends Pharmacol. Sci.* **10**:64–69 (1989).
10. Okayama, H., M. Kawaichi, M. J. Brownstein, F. Lee, T. Yokota, and K. Arai. High efficiency cloning of full-length cDNA: construction and screening of cDNA expression libraries for mammalian cells. *Methods Enzymol.* **154**:3–28 (1987).
11. Aviv, H., and P. Leder. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. USA* **69**:1408–1412 (1972).
12. Sumikawa, K., M. Houghton, J. C. Smith, J. Bell, B. M. Richards, and E. A. Barnard. The molecular cloning and characterization of a cDNA coding for the alpha subunit of the acetylcholine receptor. *Nucleic Acid Res.* **10**:5809–5822 (1982).
13. Davis, L. G., M. D. Dibner, and J. F. Battey. *Basic Methods in Molecular Biology*. Elsevier, New York, 143–145 (1986).
14. Tu, C.-P. D., and S. N. Cohen. 3-End labeling of DNA with [$\alpha$-$^{32}$P]cordycepin-5′-triphosphate. *Gene* **10**:177–183 (1980).
15. Coleman A. Translation of eukaryotic messenger RNA in *Xenopus* oocytes, In *Transcription and Translation: A Practical Approach* (D. Hames and S. Higgins, eds.). IRL, Oxford, UK, 271–300 (1984).
16. Williams, J. A., D. J. McChesney, M. C. Calayag, V. R. Lingappa, and C. D. Logsdon. Expression of receptors for cholecystokinin and other $Ca^{2+}$ mobilizing hormones in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **85**:4939–4943 (1988).
17. McIntosh, R. P., and K. J. Catt. Coupling of inositol phospholipid hydrolysis to peptide hormone receptors expressed from adrenal and pituitary mRNA in *Xenopus laevis* oocytes. *Proc. Natl. Acad. Sci. USA* **84**:9045–9048 (1987).
18. Togo, J., R. M. Burch, C. J. DeHaas, J. R. Connor, and L. R. Steranka. D-Phe-7-substituted peptide bradykinin antagonists are not substrates for kininase II. *Peptides* **10**:109–112 (1989).
19. Snutch, T. P. The use of Xenopus oocytes to probe synaptic communication. *Trends Neurosci.* **11**:250–256 (1988).
20. Snell, P. H., and C. R. Snell. Solubilization and characterization of the bradykinin receptor from rat uterus. *Biochem. Soc. Trans.* **17**:569–570 (1989).
21. Symington, P. R., A. Ashford, and G. S. Bailey. Effect of bradykinin and insulin on glucose oxidation in adipocytes from normal and streptozotocin diabetic rats. *Biochem. Soc. Trans.* **16**:1012–1013 (1988).
22. Jones, T. H., B. L. Brown, and P. R. M. Dobson. Bradykinin stimulates phosphoinositide metabolism and prolactin secretion in rat anterior pituitary cells. *J. Mol. Endocrinol.* **2**:47–53 (1989).
23. Baird, A. W., and H. S. Margolius. Bradykinin stimulates electrogenic bicarbonate secretion by the guinea pig gallbladder. *J. Pharmacol. Exp. Ther.* **248**:268–272 (1989).
24. Bascands, J. L., C. Pecher, G. Cabos, and J. P. Girolami. B2-kinin receptor like binding in rat glomerular membranes. *Biochem. Biophys. Res. Commun.* **158**: 99–104 (1989).
25. Slivka, S. R., and P. A. Insel. Phorbol ester and neomycin dissociate bradykinin receptor-mediated arachidonic acid release and polyphosphoinositide hydrolysis in Madin-Darby canine kidney cells. *J. Biol. Chem.* **263**:14640–14647 (1988).
26. Recht, L. D., and G. M. Abrams. Neuropeptides and their role in nociception and analgesia. *Neurol. Clin.* **4**:833–852 (1986).
27. Steranka, L. R., D. C . Manning, C. J. DeHaas, J. W. Ferkany, S. A. Borosky, J. R. Connor, R. J. Vavrek, J. M. Stewart, and S. H. Snyder. Bradykinin as a pain mediator: receptors are localized to sensory neurons, and antagonists have analgesic action. *Proc. Natl. Acad. Sci. USA* **85**:3245–3249 (1988).
28. Farmer, S. G., R. M. Burch, S. A. Meeker, and D. E. Wilkins. Evidence for a $B_2$ bradykinin receptor. *Mol. Pharmacol.*, **36**:7–8 (1989).
29. Higashida, H., R. A. Streaty, W. Klee, and M. Nirenburg. Bradykinin-activated transmembrane signals are coupled via $N_0$ or $N_i$ to production of inositol 1,4,5-trisphosphate, a second messenger in NG108-15 neuroblastoma-glioma hybrid cells. *Proc. Natl. Acad. Sci. USA* **83**:942–946 (1986).
30. Francel, P. C., R. J. Miller, and G. Dawson. Modulation of bradykinin-induced inositol triphosphate release in a novel neuroblastoma × dorsal route ganglion sensory neuron cell line (F-11). *J. Neurochem.* **48**:1632–1639 (1987).
31. Kaneko, S., K.-I. Kato, S.-I. Yamagishi, H. Sugiyama, and Y. Nomura. GTP-binding proteins $G_i$ and $G_o$ transplanted onto *Xenopus* oocyte by rat brain messenger RNA. *Mol. Brain Res.* **427**:11–19 (1987).
32. Burch, R. M. Bradykinin signal transduction. *J. Cardiovasc. Pharmacol.*, in press, (1990).


**Send reprint requests to:** Lawrence C. Mahan, Laboratory of Cell Biology, National Institute of Mental Health, Building 36, Room 3A09, Bethesda, MD 20892.