Guanine Nucleotide Regulation of B2 Kinin Receptors

TIME-DEPENDENT FORMATION OF A GUANINE NUCLEOTIDE-SENSITIVE RECEPTOR STATE FROM WHICH [3H]BRADYKININ DISSOCIATES SLOWLY*

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We have examined the binding of [3H]bradykinin to bovine myometrial membranes and assessed its sensitivity to guanine nucleotides. Total binding displayed a typical B2 kinin receptor specificity. However, saturation binding isotherms were resolved into at least two components with K_d values of 8 pm (45%) and 378 pm (55%). Low affinity binding exhibited relatively rapid rates of association (k_a = 1.40 \times 10^{-2} s^{-1}) and dissociation (k_d = 3.82 \times 10^{-8} s^{-1}), while high affinity binding exhibited considerably slower rates (k_a = 9.52 \times 10^{-3} s^{-1} and k_d = 4.43 \times 10^{-4} s^{-1}). Pre-equilibrium dissociation kinetics revealed that formation of high affinity binding was characterized as a time-dependent accumulation of the slow dissociation rate at the expense of at least one other more rapid dissociation rate. In the presence of 10 μM guanyl-5'-yl imidodiphosphate (Gpp(NH)p), at least two binding components were resolved with K_d values of 37 pm (12%) and 444 pm (88%). Gpp(NH)p apparently specifically perturbed high affinity binding by completely preventing the accumulation of the slow dissociation phase. Instead, two more rapid dissociation rates (k_d = 8.53 \times 10^{-3} s^{-1} and 4.43 \times 10^{-4} s^{-1}) were observed. These results suggest that [3H]bradykinin interacts with at least two B2 kinin receptor-like binding sites in bovine myometrial membranes. A three-state model for the guanine nucleotide-sensitive agonist interaction with the high affinity binding sites is proposed.

Kinins, a group of endogenous vasoactive peptides with remarkable pharmacological potency, have been implicated in a number of physiological as well as pathophysiological conditions. Kinins contract and relax a number of smooth muscles of both vascular and nonvascular origin (1). At the cellular level, kinins stimulate inositol phospholipid turnover and intracellular Ca^{2+} mobilization (2-6), prostaglandin release (7, 8), and promote both membrane depolarization and hyperpolarization (9, 10).

Receptors for kinins have been divided into two main subtypes designated B1 and B2 (1). The B2 receptor subtype is the most prevalent form of the two and appears to mediate most kinin actions under nonpathological conditions. B2 kinin receptors have been identified in vitro on intact cells (11-13) and in various membrane preparations (14-16) and in a soluble preparation (14) using either [3H]BK or Tyr-BK. The identification of the B1 kinin receptor subtype at the cellular and molecular level has been hampered primarily by a lack of high affinity radioligands and a reliable source of receptors.

Considerable interest has emerged regarding the existence of B2 kinin receptor heterogeneity. Physiological studies have revealed biphasic actions of BK (18, 19). Furthermore, some B2 receptor-specific antagonists were shown to display tissue selectivity (20-22). In addition, Manning et al. (16) presented evidence for B2 receptor heterogeneity by showing that [3H]BK binds to membranes of various guinea pig tissues with multiple affinities.

A number of agonists bind to their respective receptors in a guanine nucleotide-sensitive manner. This sensitivity is due to the presence of a guanine nucleotide regulatory protein (G-protein) as an integral component of the receptor-effector complex with high affinity for guanine nucleotides and that serves to transduce the signal elicited by agonist binding from the receptor to the effector component (23). Several reports suggest that both hormone-promoted polyphosphoinositide hydrolysis and release of arachidonic acid metabolites are regulated by specific G-proteins (24, 25). The properties of these putative G-proteins are presently unknown. Kinin peptides stimulate both of the above cellular responses. Consequently, we were interested in evaluating the effect of guanine nucleotides on agonist binding to B2 kinin receptors to determine if a G-protein may be involved in B2 kinin receptor signaling.

Here, we show that the agonist [3H]BK recognized two binding sites with B2 kinin receptor specificity in bovine myometrial membranes. These sites were distinguished based on their distinct equilibrium binding affinity for the radiolabeled agonist. High affinity [3H]BK binding involved a sequential formation of binding states with decreasing dissociation rates for [3H]BK. This sequence was interrupted by guanine nucleotides. These results suggest that high affinity bovine myometrial B2 kinin receptors interact with a G-protein.

EXPERIMENTAL PROCEDURES AND RESULTS

We have examined the characteristics of the binding of the B2 kinin receptor agonist [3H]BK to bovine myometrial membranes.

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1 Portions of this paper (including "Experimental Procedures," "Results," Tables 1 and 2, and Figs. 1-10) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
branes and assessed its sensitivity to guanine nucleotides. The experimental procedures and results are described in the Miniprint. At equilibrium [3H]BK interacted with these membranes with at least two affinities. Furthermore, the total specific binding displayed a typical B2 kinin receptor specificity. These results indicate that at least two distinct B2 kinin receptor-like binding sites may be present in this tissue. The interaction of [3H]BK with the high affinity binding sites involved a guanine nucleotide-sensitive sequential formation of binding states with decreasing dissociation rates for [3H]BK. The presence of multiple binding states of the high affinity B2 kinin receptor-binding sites may in part be a consequence of coupling to a guanine nucleotide regulatory protein.

The data presented suggests that the low affinity bovine myometrial B2 kinin receptor sites exist in a single binding state (Equation 1) and the high affinity receptor sites exist in at least three binding states (Equation 2). One model for the formation of such states for each receptor site is described below

\[
L + R_L \rightarrow LR_L, K_L
\]

\[
L + R_R \rightarrow LR_R, K_R
\]

\[
L + R_{LR} \rightarrow LR_{LR}, K_{LR}
\]

where BK (L) interacts with the low affinity receptor (R_L) to yield one complex (LR_L) and with the high affinity receptor (R_R, R_{LR} or R_{LR*}) to yield three complexes (LR_R, LR_{LR}, or LR_{LR*}). K_L represents the equilibrium association constant for the interaction with the low affinity receptor and K_R = K_{LR}, represents equilibrium association constants for the interaction with the high affinity receptor. The following discussion is the rationale for proposing the above models.

The high and low affinity binding sites were studied concomitantly using approximately 2 nM [3H]BK, and the high affinity binding sites were studied essentially independently of the low using approximately 0.05 nM [3H]BK. Using the experimental K_d values of 8 and 378 pM, respectively, we estimated that under control conditions 100% of the high affinity sites and 84% of the low affinity sites were occupied at 2 nM [3H]BK and 84% of the high affinity sites and 12% of the low affinity sites were occupied at 0.05 nM [3H]BK.

At 2 nM [3H]BK, the association and dissociation kinetics were biphasic. On the other hand, at 0.05 nM [3H]BK the kinetics were monophasic and the rate of dissociation of equilibrium binding and the rate of association were not significantly different from the slow rates of dissociation and association, respectively, observed at 2 nM [3H]BK. Thus, we assumed that the binding of [3H]BK to the low affinity sites exhibited fast rates (k_{obs} = 1.40 \times 10^{-2} \text{ s}^{-1}, k_{-1} = 3.82 \times 10^{-4} \text{ s}^{-1}) and the binding to the high affinity sites exhibited slow rates (k_{obs} = 8.59 \times 10^{-4} \text{ s}^{-1}, k_{-1} = 4.43 \times 10^{-5} \text{ s}^{-1}). Manning et al. (16) determined that [3H]BK binds to guinea pig ileum membranes with K_d values of 13 and 910 pM. These values are similar to those obtained here and suggest that the same B2 kinin receptor subtype were identified. In contrast, this group assigned the rapid association rate to the high affinity sites. As described above, it is clear that such an assignment cannot be made with [3H]BK binding to the bovine myometrial B2 kinin receptor sites that we observed.

The value of the observed association rate constant did not vary between 0.005-0.05 nM [3H]BK and was not significantly different from that observed for the slow rate constant at 2 nM [3H]BK. These results indicate that the binding of [3H]BK to the high affinity receptor sites may proceed via multiple affinity states rather than via a simple bimolecular reaction. The presence of more than one binding state of the high affinity site is supported by the fact that pre-equilibrium binding assayed with 0.05 nM [3H]BK dissociated with multiple rates. Two rates are clearly distinguishable. One of the rates is relatively slow and corresponds closely to the slow rate observed at equilibrium. The other rate is fast and is quite similar to the dissociation rate of the high affinity state of the low affinity sites. In order to determine the order whereby these rates appear, we assayed the dissociation kinetics at various stages of [3H]BK association. These studies revealed that at 10 s all binding dissociated with the fast rate. In a time-dependent manner, the slow rate accumulated and reached a maximum level of 64 and 100% of the total binding when assayed at 2 and 0.05 nM [3H]BK, respectively. Thus, the formation of the equilibrium binding state of the high affinity sites, termed LR_{LR*}, which exhibits a relatively slow dissociation rate, appears to be dependent on the formation of at least one transient pre-equilibrium binding state which exhibits a relatively fast dissociation rate. No changes in the low affinity component of the dissociation kinetics were apparent. Furthermore, all the time-dependent changes in the dissociation kinetics observed at 2 nM [3H]BK were also observed using 0.05 nM [3H]BK. Thus, we assume that the low affinity binding sites form a single homogeneous binding state (LR_{LR}).

The use of guanine nucleotides provided significant additional information regarding the characteristics of agonist binding to the high affinity binding sites. Even though conclusive evidence would require an assay of the low affinity sites without the interference by the high affinity sites, we did not observe any effect of guanine nucleotides on the binding of [3H]BK to the low affinity sites. When the high affinity sites were assayed essentially independently of the low affinity sites, we observed that dissociation of equilibrium binding of [3H]BK proceeded with two phases in the presence of guanine nucleotides rather than with one phase as observed in the absence of guanine nucleotides. The rate of one of these two phases (k_{-1} = 3.83 \times 10^{-4} \text{ s}^{-1}) is similar to the fast rate observed prior to equilibrium in the absence of guanine nucleotides (k_{-1} = 3.82 \times 10^{-2} \text{ s}^{-1}). The other phase has a rate (k_{-1} = 4.43 \times 10^{-4} \text{ s}^{-1}) of a magnitude in between that of the fast (k_{-1} = 3.82 \times 10^{-2} \text{ s}^{-1}) and slow (k_{-1} = 4.43 \times 10^{-5} \text{ s}^{-1}) rates. Interestingly, the shallowness of pre-equilibrium dissociation in the absence of guanine nucleotides suggests the presence of other transient pre-equilibrium dissociation phase with values between that of the fast and slow rates and similar to that of the slow dissociation rate observed in the presence of guanine nucleotides. These results support the presence of two pre-equilibrium binding states of the high affinity sites. One state, termed LR_{LR*}, exhibits a relatively fast dissociation rate, and a second state, termed LR_{LR}, exhibits a dissociation rate between that of the fast and slow rates and accumulates
only in the presence of guanine nucleotides. Thus, guanine nucleotides completely prevent the formation of LR$i*$. On the other hand, LR$&*$ and LR$g*$ can still form.

In the presence of guanine nucleotides [3H]BK binds to myometrial membranes with two affinities (37 and 444 pm). The relative level of the higher affinity binding observed in the presence of guanine nucleotides (12%) does not correspond to that of high affinity binding observed in the absence of guanine nucleotides (45%). The dissociation kinetics indicate that in the presence of guanine nucleotides a significant portion of the high affinity binding sites exist in a rapidly dissociating state (LR$i*$). Thus, based on the above assumption, the high and low affinity binding observed in the presence of guanine nucleotides probably represent LR$n*$ and LR$i* + LR&_*$ respectively. Following this assumption, 12% of the total binding exists as LR$n*$. As indicated by the dissociation kinetics, the residual portion of the high affinity binding must represent a binding state with rapid dissociation kinetics. This is supported by the presence of a significant amount of rapidly dissociating binding in the presence of guanine nucleotides. Since we cannot clearly distinguish LR$n*$ and LR$n_*$, we cannot directly determine the relative level of LR$n*$.

At 0.05 nM, Gpp(NH)p maximally inhibited binding approximately 60%. Using the experimental values of the relative levels of the high and low affinity binding sites and the $K_D$ values for each of the receptor states, at 0.05 nM [3H]BK 66% of the binding should be sensitive to guanine nucleotide. This value is close to the experimental value above and shows that the kinetic parameters determined for each binding site are essentially correct.

In summary, the apparent monophasic dissociation kinetics of BK binding to $R_t$ suggest that $R_t$ forms a single binding state (Equation 1). The lack of dependence of the association rate on low concentrations of BK indicates that the high affinity binding reaction is complex and involves multiple kinetic states. One possibility is that the interaction of BK and the receptor initially yields a binding complex(es) from which BK dissociates rapidly and which we have termed LR$n*$ and LR$i*$. As described in Equation 2, in a time-dependent manner the complex becomes stabilized through rapid conversion of LR$i*$ and LR$n*$ to a state from which BK dissociates slowly (LR$i*+$). For LR$i*$ this conversion proceeds via LR$i*$. Increasing the time of BK exposure favors the formation of LR$i*+$ at the expense of LR$n*$ and LR$i*$ and after 1 min at 25 °C LR$i*+$ represents approximately 41% of the total binding and at equilibrium (90 min at 25 °C) 100% of the total binding. Addition of guanine nucleotides prior to or concomitantly with BK prevents the formation of LR$i*+$. Instead, both LR$n*$ and LR$i*$ accumulates in a ratio of approximately 1:1. [3H]BK binding to LR$i*$ shows that $K_D > K_{HR}$ and that $K_{HR} > K_{HRH}$, and that $K_{HRH} > K_{HR}$ and $K_{HR} > K_{HRH}$. Hence, the sequence LR$n*$ → LR$i*$ → LR$i*+$ is favorable. We have not been able to determine if any LR$i*$ is available to bind BK or if the formation of LR$i*+$ is completely dependent on prior formation of LR$i*$. However, LR$i*+$ is clearly in equilibrium with the medium as it exhibits a dissociation rate distinct from LR$n*$ and LR$i*$.

These results suggest that LR$i*$ probably represents the state of the high affinity bovine myometrial 2$B$ kinin receptor that is effectively coupled to a G-protein.

Another model is where LR$n*$ represents an alternative state that LR$i*$ partially proceeds to and equilibrates with in the absence of LR$i*$ formation (in the presence of guanine nucleotides) rather than an intermediate state in the sequence of events leading to formation of LR$i*$. In the absence of evidence for triphasic pre-equilibrium dissociation curves in the absence of guanine nucleotides, this model is also likely.

A two-state model has been used to describe the interaction of agonists with a number of receptors (29–33). Our results suggest that a third binding state (LR$n*$) is involved in BK binding to high affinity 2$B$ kinin receptor. We do not yet know the role of such a putative state. One possible explanation is that this represents a state of the receptor which is coupled to a G-protein but in a manner that does not promote an effective functional outcome. Such a state may be required for the ultimate effective functional coupling of the receptor and the G-protein. An analogous state has been proposed for the myocardial muscarinic receptor-G-protein complex (34). Interestingly, detergent extraction of guanine nucleotide-sensitive [3H]BK binding does not require prior exposure of membranes to Bradykinin. Hence, a "pre-coupled" form of the receptor may either exist or may easily form in the membrane under some conditions.

Based on dissociation rate constants as well as apparent equilibrium dissociation constants for [3H]BK, HR$n*$ and HR$i*$ may have similar binding characteristics. Furthermore, both high and low affinity binding sites display typical 2$B$ kinin receptor specificities. We have no information about the biochemical differences between the high and low affinity sites. However, we know from sucrose gradient sedimentation studies of soluble binding activity that the sedimentation rate of at least a portion of the molecular species that [3H]BK binds to in bovine myometrial membranes decreases following exposure to Gpp(NH)p. It is conceivable that the high and low affinity binding site represent forms of $B$2 receptors that are able and unable, respectively, to couple to a G-protein.

The two binding sites may represent distinct subtypes of the $B$2 receptor, and their presence in our preparation may be a consequence either of their coexistence in bovine myometrial tissue or from the fact that one of them exists in a tissue, e.g. endometrium, that is contaminating our preparation. The presence of $B$2 bradykinin receptor subtypes has been proposed (16, 18–22).

Odyt et al. (35) reported that the angiotensin converting enzyme/kininase II inhibitor SQ20,881 inhibited $^{125}$I-Tyr$^*$. BK binding to pig renal medulla membranes, suggesting that binding in these membranes involves the above BK-degrading enzyme. To test if any of the two binding sites identified here is the angiotensin converting enzyme/kininase II enzyme, we included either SQ20,881 or angiotensin I, a peptide inhibitor and a peptide substrate for this enzyme, respectively. Neither of these peptides interfered with [3H]BK binding to bovine myometrial membranes. Thus, we have excluded the possibility that any of the binding sites identified in bovine myometrial membranes is the angiotensin converting enzyme/kininase II enzyme.

A scenario similar to the one that we have described for the bovine myometrial 2$B$ kinin receptor has been described for some guanine nucleotide-sensitive receptor systems such as the adrenergic (33), muscarinic cholinergic (34), $\delta$ opioid (36) and has been proposed to be a consequence of a coupling of these receptors with a G-protein. Agonist-promoted inositol phospholipid metabolism and arachidonic acid release has been proposed to involve a G-protein. Cholera toxin and pertussis toxin, which ADP-ribosylate specific G-proteins, have served as tools to probe the involvement of G-proteins in these processes. $B$K-promoted inositol phosphate production and arachidonic acid release in A431 epidermoid carcinoma cells (4), bovine pulmonary artery endothelial cells (6), and Swiss 3T3 fibroblasts (8) were not sensitive to pertussis toxin while bradykinin-promoted hyperpolarization and de-
polarization in NG108-15 neuroblastoma-glioma hybrid cells were inhibited (2). Thus, the B2 kinin receptor may be linked to different G-proteins depending on the cell type which it is located in and/or which second messenger system it is associated with.

We have shown that bovine myometrial membranes contain high and low affinity binding sites for \[^3H\]BK which display a pharmacological specificity typical of a B2 kinin receptor. We propose that formation of a G-protein-receptor coupling involves the sequential formation of at least three receptor-binding states with decreasing dissociation rates for \[^3H\]BK.

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Guanine Nucleotide Regulation of B2 Kinin Receptors

Effect of guanine nucleotides on [3H]BHK binding to B2 kinin receptors in bovine myocardial membranes

Equilibrium binding of [3H]BHK to B2 kinin receptors in bovine myocardial membranes

[3H]BHK interacted with a finite number of binding sites in bovine myocardial membranes. Scatchard analysis of binding data revealed that equilibrium binding of [3H]BHK bound to these membranes with 1 site per molecule. The Scatchard plot was not a straight line. The sites were blocked by various unlabeled peptides with a typical B2 kinin receptor specificity. As shown in Fig. 3, the specific high affinity binding was essentially abolished in the presence of 10 μM unlabeled peptide. Although the sites at low affinity were not affected, this site was blocked by a 10 μM unlabeled peptide. The 3 H kinin receptor-selective antagonist HOE 969 did not affect the high affinity binding. However, the 3 H kinin receptor-selective antagonist HOE 969 did not affect the high affinity binding. However, the 3 H kinin receptor-selective antagonist HOE 969 did not affect the high affinity binding. However, the 3 H kinin receptor-selective antagonist HOE 969 did not affect the high affinity binding.
Guanine Nucleotide Regulation of B2 Kinin Receptors

![Graph](image)

Fig. 3. Competition of specific [125I]BHK binding with kinin-related peptides in bovine myocardial membranes. A suspension of 9 pmol (approx. 0.1 nmol) of 125I-BHK was incubated with bovine myocardial membranes and with increasing concentrations of kinin peptides as shown and the binding assays performed as described under "Experimental Procedures." The results are presented as % of Control where control refers to specific [125I]BHK binding to membranes in the absence of any cascade. The experiments were done in triplicate and the result is the average of two experiments that differed ±12.

![Graph](image)

Fig. 4. Association kinetics of specific [125I]BHK binding to bovine myocardial membranes at various [125I]BHK concentrations. The rate of association of 125I-BHK binding to membranes was measured as the rate of formation of 125I-BHK binding to membranes at various [125I]BHK concentrations. The data are presented as a pseudo-first-order kinetic plot. The scope of each phase is equal to 1/k, the observed rate constant for the pseudo-first-order reaction. Nonspecific binding is essentially maximal at the first time point (0.2% M). Each assay point is the average of duplicate determinations and the result in (B) is the average ± S.E.M. of four experiments and the result in (B) is the average of two experiments that differed ±12.

![Graph](image)

Fig. 5. Dissociation kinetics of specific [125I]BHK binding to bovine myocardial membranes at various [125I]BHK concentrations. Bovine myocardial membranes were incubated with approx. 2 nmol [125I]BHK and approx. 0.05 nmol [125I]BHK for 90 min. Dissociation was initiated by 5-fold dilution and incubating the suspension at 37°C. The samples were incubated at 37°C for 10 min and then the density of protein was measured by radioactivity. Nonspecific binding is essentially maximal at the first time point (0.2% M). Each assay point is the average ± S.E.M. of four experiments and the result in (B) is the average of two experiments that differed ±12.

![Graph](image)

Fig. 6. Effect of various association times on the dissociation kinetics of specific high and low affinity [125I]BHK binding to bovine myocardial membranes. Bovine myocardial membranes were incubated with approx. 2 nmol [125I]BHK for 0.08 min. (A), 0.1 min. (B), 0.15 min. (C), 0.2 min. (D), 0.25 min. (E), 0.5 min. (F), 1 min. (G), 2 min. (H), 4 min. (I), 10 min. (J), 20 min. (K), 40 min. (L). Other conditions were as described in Fig. 5. Each assay point was performed in duplicate and each curve is the average of two experiments.

![Graph](image)

Fig. 7. Effect of various association times on the dissociation kinetics of specific high affinity [125I]BHK binding to bovine myocardial membranes. Bovine myocardial membranes were incubated with approx. 0.05 nmol [125I]BHK for 1 min. (A), 2 min. (B), 20 min. (C). Other conditions were as described in Fig. 5. Each assay point was performed in duplicate and each curve is the average of two experiments.

### TABLE 1

| Condition | Low Affinity | Rapid | Intermediate | Slow |
|-----------|--------------|-------|---------------|------|
| Control   | 392 ± 24     | ---   | ---           |      |
| + 10 μM Gpp(NH)p | 417 ± 37     | 653 ± 195 | 44.3 ± 4.6    | --- |

* Low and high affinity [125I]BHK binding was assayed using approx. 2 nmol and 0.05 nmol [125I]BHK, respectively, as described in Fig. 5. The results are presented as mean ± S.E.M. of three to five separate experiments with each assay performed in duplicates.
**Fig. 2.** Effects of Gpp(NH)p on the dissociation kinetics of specific high affinity $[^3]H$HN binding to bovine myometrial membranes. Bovine myometrial membranes were incubated with approx. 0.43 pM $[^3]H$HN for 5 min., W.A.) and 90 min. (C) in the absence (filled symbols) and presence (open symbols) of 150 μM Gpp(NH)p. Other conditions were as described in Fig. 2. Specific $[^3]H$HN binding at 5 min is 41.2 ± 2.9% of equilibrium binding (163.8 ± 15.0 fmol/mg of protein). Each assay point was performed in duplicate and each curve is the average of two experiments.

**Fig. 3.** Effects of Gpp(NH)p on the dissociation kinetics of specific high and low affinity $[^3]H$HN binding to bovine myometrial membranes. Bovine myometrial membranes were incubated with approx. 2 nM $[^3]H$HN for 50 min. Dissociation was initiated by 5-fold dilution and making the suspension of 1 μM ER and 10 μM NMA. The results are presented in Fig. 5. Each assay point was performed in duplicate and the results are shown as average ± S.E.M. of three to five experiments.

**Table 2**

| Treatment          | Specific $[^3]H$HN Binding (%) |
|--------------------|-------------------------------|
| Control            | 100                           |
| + 10 μM GTP        | 24                            |
| + 10 μM Gpp(NH)p   | 34                            |
| + 10 μM ATP        | 59                            |
| + 10 μM App(NH)p   | 94                            |

*Membranes were dialyzed for 30 min, with 2 μM $[^3]H$HN. Dissociation was then initiated by addition of BR (1 μM final concentration); in the absence (Control) or presence of various nucleotides and ammonium allowed to proceed for 30 min, before assays were terminated. Values are averages of two experiments that differed <10%.

**Fig. 4.** Effects of Gpp(NH)p on the dissociation kinetics of specific high affinity $[^3]H$HN binding to bovine myometrial membranes. Bovine myometrial membranes were incubated with approx. 0.05 μM $[^3]H$HN for 50 min. In the presence of varying concentrations of Gpp(NH)p (W.A.) and App(NH)p (W.A.). Assays were performed essentially as described in Fig. 2. 14% specific $[^3]H$HN binding is 175 ± 6.4 fmol/mg of protein (mean ± S.E.M., n = 11) and total/nonspecific binding is as shown in Fig. 2. Each assay point was performed in duplicate and the results are shown as average ± S.E.M. of four experiments.
Guanine nucleotide regulation of B2 kinin receptors. Time-dependent formation of a guanine nucleotide-sensitive receptor state from which [3H]bradykinin dissociates slowly.

L M Leeb-Lundberg and S A Mathis

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