Biochemical Pharmacology, Vol. 39, No. 12, pp. 1921–1928, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

CHARACTERIZATION OF BRADYKININ RECEPTORS SOLUBILIZED FROM RAT UTERUS AND NG108-15 CELLS

PENELOPE H. SNELL, ELSA PHILLIPS, GILLIAN M. BURGESS, CHRISTOPHER R. SNELL* and MICHAEL WEBB

Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN, U.K.

(*Received* 7 *June* 1989; *accepted* 18 *January* 1990)

Abstract—In this paper we report the solubilization of bradykinin B2 binding sites from membranes prepared from NG 108-15 tumours and rat uterus with retention of binding activity. Digitonin was found to solubilize the receptors from both tissues, and the addition of CHAPS increased the yield of soluble receptor from rat uterus only. The affinity of a range of bradykinin analogues has been shown to have the same rank order for both the soluble and membrane receptors from both tissues, and in corresponding functional assays. In addition, the binding of bradykinin ligands to the soluble and membrane receptors is similarly modulated by the presence of sodium ions. We conclude that the soluble binding sites correspond to the physiological bradykinin B2 receptor present in these tissues.

Kinins are peptide hormones which are known or suspected to be involved in a range of physiological and pathological processes. These include vasodilatation, the control of vascular permeability, inflammation and pain sensation [1–4]. They have also been found to act as mitogens in cultured fibroblasts [5]. This diversity of function is reflected in the widespread distribution of kinin receptors in various tissues, including smooth muscle, brain, and on the terminals of primary afferent nociceptive neurons [2, 6–9]. The characterization of these receptors at the biochemical level may be expected to clarify their mode of activation and desensitization, their interactions with signal transducing proteins, and the possible existence of multiple receptor subtypes. An important preliminary requirement is the solubilization of kinin receptors which retain their ligand binding properties in solution. Only two reports of such studies have appeared so far [10, 11], and these describe the characterization of soluble bradykinin binding sites from bovine uterine membranes. We now describe our own studies on the bradykinin binding activity solubilized from two sources: (i) rat uterus; and (ii) tumours grown from the neuroblastoma × glioma hybrid cell line NG 108-15. We show that the binding activity solubilized from both sources resembles that seen in the intact membranes and appears to represent truly solubilized receptors.

MATERIALS AND METHODS

Sources

[^{3}H]Bradykinin was purchased from Amersham International (Amersham, U.K.), Digitonin was purchased from Wako Chemical Co. (Tokyo, Japan). ACE, enzyme inhibitors and other detergents were purchased from the Sigma Chemical Co. (Poole, U.K.) and Calbiochem Inc. (La Jolla, CA). Iodo-Tyr^{1}Kallidin was kindly synthesized by Dr H. deVries (Sandoz, London, U.K.).

Cells and cell culture

NG 108-15 hybrid cells [12] were used from two different sources, either of known passage number up to P30, or of unknown passage number. No qualitative or quantitative differences were found between these two populations. Cells were maintained in Dulbecco's modified Eagles medium supplemented with 10% (v/v) foetal calf serum and penicillin/streptomycin at 37°. They were passaged at ratios of 1:5 or 1:10 approximately twice weekly. Cells were harvested mechanically by vigorous shaking of the culture vessel.

Production of tumour membranes

Approximately 10^6 NG 108-15 cells were injected subcutaneously in a volume of 100 μL into each of two sites on the back of nude mice, and tumours were harvested approximately 6 weeks later. They were homogenized in 25 mM potassium phosphate, pH 6.5, at 4° using a polytron (setting 9, 1 min) and the particulate fraction was spun down at 40,000 *g* (30 min) and washed four times by resuspension in the same buffer. This particulate fraction (crude tumour membranes) was finally resuspended at 7 mg/mL in the same buffer, and aliquots were frozen and stored at −70°.

Preparation of uterine membranes

Uteri were removed from female rats 18–24 hr after priming with stilboestrol. The tissue was roughly chopped before homogenization in 25 mM potassium phosphate buffer, pH 6.5, using a Polytron tissue disrupter (setting 9 for 45 sec). The crude particulate preparation was washed three times by successive centrifugation at 40,000 *g* (30 min) and

* To whom correspondence should be addressed.

† Abbreviations: CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate; CHAPSO, 3-[(3-cholamidopropyl)-dimethylammonio]2-2-hydroxy-1-propane sulfonate; HEPES, *N*-2-hydroxyethyl-piperazine-*N*'-2-ethanesulfonic acid; EGTA, ethyleneglycol-bis-(β-aminoethyl)-*N*,*N*,*N*',*N*'-tetaacetic acid; HPLC, high pressure liquid chromatography; ACE, angiotensin converting enzyme.

rehomogenization. The final membrane preparation was frozen in aliquots in the above buffer at −70° for subsequent use.

$^{45}Ca^{2+}$ efflux assay

The agonist activity of a series of bradykinin analogues was assessed by measuring the increase in the rate of efflux of $^{45}Ca^{2+}$ from preloaded NG 108-15 cells [13]. NG 108-15 cells were grown on Terasaki plates (500 cells/well) in Dulbecco's modified Eagles medium containing 2% foetal calf serum and 1 mM dibutyryl cyclic AMP for 5 or 6 days. The cells were loaded with $^{45}Ca^{2+}$ in growth medium (10 μL/well; radioactive concentration = 20 μCi/mL) for 2 hr at 37°.

Each Terasaki plate was washed with HEPES buffered salt solution (7 × 8 mL) over a period of 20 min at 37° to remove excess $^{45}Ca^{2+}$. The experiment was then conducted as follows: the plate was washed with 8 mL of the above buffer at 1 min intervals, and at the seventh min the agonist was added for a period of 1 min. After removal of the medium containing the agonist, the cells were washed with a further four changes of medium at 1 min intervals. Each of the 11 × 8 mL washes was collected for scintillation counting. At the end of the experiment, the cells were solubilized in 0.2% SDS and the remaining $^{45}Ca^{2+}$ counted. A single plate (total contents 30,000 cells) was used for each experimental determination of Ca^{2+} efflux.

The data was analysed by calculating a rate coefficient, defined as $(\Delta C/\Delta T)^* C_m$ where ΔC is the amount of radioactivity lost from the cells during each collection period of duration ΔT and C_m is the mean of $^{45}Ca^{2+}$ content of the cells at time T and $T + \Delta T$. The rate constant was calculated for the interval at 1 min after adding the agonist.

Preparation of [^{125}I]Tyr^1.kallidin

Tyr^0.bradykinin was iodinated using the method of Hunter and Greenwood [14]. Briefly, Tyr^1.kallidin (4 μg) was added to 1 mCi of Na^{125}I in 0.25 M potassium phosphate buffer, pH 7.4 (20 μL), followed by chloramine T (20 μL, 1 mg/mL). After 3 min at room temperature, sodium metabisulphite (20 μL, 1 mg/mL) was added, and the reaction mixture was combined with a methanol wash (100 μL). It was immediately chromatographed on HPLC (C_{18} reverse phase; gradient 95% A to 65% A: A = 0.5% ammonium acetate, B = acetonitrile). The peak corresponding to monoiodinated Tyr^1.kallidin was collected, stored at 4° and used within a period of 2 weeks; sp. act. 2200 Ci/mmol.

Solubilization of crude membrane preparations

Uterine membranes. Detergent solubilization and assay of detergent soluble extracts was carried out in 25 mM potassium phosphate buffer pH 6.5 containing the following enzyme inhibitors: EGTA (1 mM), bacitracin (0.1 mM), enalaprilat (4 μM), pivaloyl arginine (50 μg/mL), *ortho*-phenanthroline (1 mM) and soybean trypsin inhibitor (50 μg/mL). All procedures were carried out at 4°. Aliquots of uterine membranes were thawed, homogenized using a Polytron homogenizer, and an equal volume of buffer containing twice the final concentration of inhibitors and detergent added. The resulting suspension (10 mg membrane protein/mL) was mixed at 4° for 30 min and then centrifuged at 100,000 *g* for 60 min. The supernatant was used in the soluble binding assay without further treatment.

Tumour membranes. Detergent solubilization and assay of detergent soluble extracts was carried out in 25 mM potassium phosphate buffer pH 6.8 containing 0.2% bovine serum albumin and the following enzyme inhibitors: EGTA (1 mM) bacitracin (0.1 mM), enalaprilat (4 μM), pivaloyl arginine (50 μg/mL), *ortho*-phenanthroline (1 mM), chymostatin (50 μg/mL) and soybean trypsin inhibitor (100 μg/mL). All procedures were carried out at 4°. Aliquots of crude membranes (7 mg protein/mL) were thawed, and an equal volume of buffer containing twice the final concentration of inhibitors was added. The diluted membranes were rehomogenized in a Potter teflon/glass homogenizer, and an equal volume of solubilization buffer containing inhibitors with twice the final concentration of detergent was added. The mixture was agitated at 4° for 30 min, and then centrifuged at 120,000 *g* for 60 min. The supernatant was then used without further treatment, although in some experiments it was passed through a 0.22 μm filter prior to use.

Particulate binding assays

Uterine membranes, whole NG 108-15 cells or tumour membranes (0.25 mg protein) were added to a serial dilution of bradykinin or analogue under study in binding buffer (25 mM potassium phosphate buffer, pH 6.5, containing 0.1 mM bacitracin, 1 mM *ortho*-phenanthroline, 1 mM EGTA and 50 μg/mL of chymostatin) and [^{125}I]Tyr^1.kallidin (100,000 cpm; 45 pM), or [^{3}H]bradykinin (500 pM), in a total volume of 1 mL. After incubation of the uterine membranes at 4° for 30 min the bound ligand was separated from free by rapid centrifugation at 14,000 *g* (3 min, 4°). Pellets were washed superficially with ice-cold buffer and the radioactivity counted. After incubation of whole NG 108-15 cells or membranes at 4° for 30 min the bound ligand was separated from free by rapid filtration using a Brandel cell harvester. Filters were soaked in 0.1 M potassium hydroxide solution (0.25 mL) for 2 hr before addition of scintillant and counting of radioactivity. Displaceable binding was taken as the difference between that bound in the absence and presence of 1 μM iodo-Tyr^1.kallidin, and was 60 and 75% of total binding for uterine and NG 108-15 cells or membranes, respectively.

Soluble binding assays

The detergent extract (200 μL) was incubated with [^{125}I]Tyr^1.kallidin (10 μL; 100,000 cpm; about 250 pM) and a serial dilution of bradykinin analogue (23 μL) in a final volume of 233 μL, at 4° for 30 min (uterine receptor) or 2 hr (NG 108-15 receptor). Bound ligand was separated from free by gel filtration on Sephadex G50 (bed volume 1.8 mL) in disposable columns. The incubation mixture (233 μL) was applied to the column and washed on with 25 mM phosphate buffer, pH 6.5, containing 0.2% bovine serum albumin, 1 mM EGTA and 0.1 mM bacitracin (100 μL). The columns were

eluted with the same buffer (1.15 mL), and the total eluate, containing the bound ligand, was collected as a single fraction, and the radioactivity counted. Unbound ligand remained on the column. Displaceable binding was taken as the difference between that bound in the absence and presence of 1 μM unlabelled iodo-Tyr^1.kallidin, and represented 85% of total binding.

The data for sodium chloride dependence and time courses for association were obtained by simple modification of the above protocols for the particulate and soluble receptor assays. For all these experiments [^{125}I]Tyr^1.kallidin was used as label with 1 μM iodo-Tyr^1.kallidin to determine the non-specific binding, except for the association time course for whole NG 108-15 cells where [^{3}H]bradykinin was used as label and 1 μM bradykinin used to determine non-specific binding.

Data processing

Binding curves and kinetic data were fitted by least-squares iteration using the method of Marquardt [15].

RESULTS

Detergent solubilization of bradykinin receptors

A variety of detergents were tested for their ability to solubilize bradykinin binding sites from rat uterus and NG 108-15 tumour membranes with retention of binding activity. The detergents tested included cholate, taurocholate, CHAPS, CHAPSO, 2-deoxybigchap, octyl glucoside, Lubrol PX, Zwittergent 3-14, Triton X 100, C12E8, and digitonin. Of these, only digitonin solubilized binding activity from both tissue sources. The yield of solubilized bradykinin receptors was dependent on the concentration of the digitonin employed, the optimum concentrations being 0.3 and 0.5% for the NG 108-15 tumour and uterine membranes, respectively. At these concentrations of digitonin approximately 20% of total membrane protein and 15% of binding activity was solubilized from both tissues. Increasing the concentration of digitonin much above this level failed to increase the yield of binding activity. After solubilization very little binding activity remained in the insoluble pellet indicating some degree of receptor inactivation, probably caused by denaturation during exposure to the detergent. The yield of soluble binding sites from uterine membranes could be increased to 30% by the use of 3 mM CHAPS in combination with 0.5% digitonin. This combination of detergents was used in all subsequent experiments with rat uterine tissue. However, this procedure did not increase the yield of receptor in digitonin extracts of NG 108-15 membranes. The binding activity in the supernatant was shown to be truly soluble using the following criteria: the binding activity remained in solution after centrifugation for 60 min at 100,000 g_{av}, and also passed freely through 0.22 μm filters.

The stability of the binding activity in the detergent extracts was assessed under various storage conditions. The tumour membrane receptor retained 40% of binding activity after storage for 24 hr at 4°. Thereafter, the rate of loss of activity appeared to be slower, and 23% of binding activity remained after 3 days at this temperature. When frozen at −30° the soluble tumour receptor lost 58 and 80% of binding activity after 24 or 48 hr, respectively. Binding activity was more stable at −70° with 87% of activity retained after 48 hr at this temperature.

The soluble uterine receptor was considerably less stable than the tumour receptor on storage at 4°, losing 80% of binding activity after 24 hr. At −20°, the soluble uterine receptor was somewhat more stable than the NG 108-15 receptor, losing 38 and 70% of binding activity after 3 and 4 days, respectively.

Association of bradykinin ligands to soluble and membrane receptors

Uterine receptor. Figure 1 shows the association time course for [^{125}I]Tyr^1.kallidin binding to uterine membrane and the solubilized uterine receptors. The association time courses are pseudo first-order with k_{obs} of 0.062 m^{-1} and 0.059 m^{-1} for the membrane and soluble receptor, respectively, with corresponding $t_{1/2}$ values of 20 and 6 min. The membrane receptor reaches equilibrium at this ligand concentration after 2 hr and remains stable for at least another hour. The soluble receptor, on the other hand, appears to reach maximal binding at around 1 hr and shows a decay of binding thereafter. This loss of binding does not correspond to degradation of the ligand (as shown using HPLC) and probably represents denaturation of the receptor. In subsequent equilibrium binding studies 2 hr and 30 min were allowed for equilibrium to be reached for the membrane and soluble receptors, respectively; the incubation time for the soluble receptor being a compromise to maximize the approach to equilibrium and minimize loss of soluble receptors to denaturation.

NG 108-15 receptor. Figure 2 shows the association time course for binding of [^{125}I]Tyr^1.kallidin and [^{3}H]bradykinin to NG 108-15 soluble and whole cell receptors, respectively. The association is pseudo first-order in both cases with k_{obs} of 0.058 m^{-1} and 0.045 m^{-1}, respectively. The $t_{1/2}$ for the whole cell receptor was 4 min with equilibrium being reached at 30 min, whereas the $t_{1/2}$ for the soluble receptor was 15 min and equilibrium was reached at 2 hr.

Displacement data for soluble and membrane receptors

The IC_{50} values for a range of bradykinin analogues have been obtained for the membrane and soluble receptors from rat uterus and NG 108-15 cells. The results of these experiments are shown in Table 1. The displacement curves are parallel to that for bradykinin with slopes compatible with one apparent binding site. In addition the table shows the EC_{50} values for relevant functional assays in the two systems, i.e. the Ca^{2+} efflux assay for NG 108-15 cells and the ability of the analogues to stimulate contraction of the rat uterus *in vitro*.

There is good correlation between the IC_{50} values for the membrane and soluble receptors from each tissue over a range of five orders of magnitude of ligand affinities (R = 0.98 and 0.93 for the NG 108-15 and uterine receptors, respectively). In general,

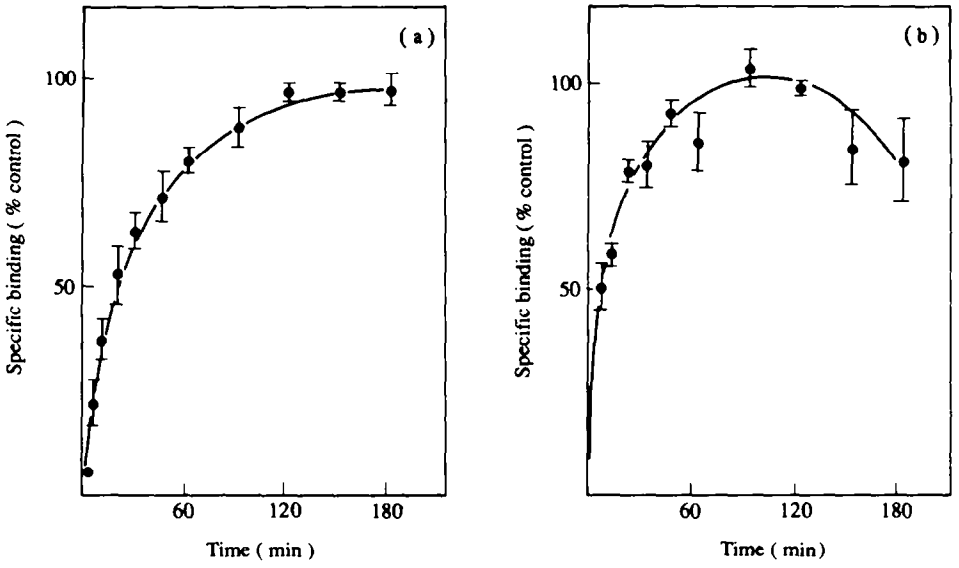

Fig. 1. Time course for association of [^{125}I]Tyr^1.kallidin to the membrane (a) and soluble (b) binding sites from rat uterus. Specific binding to membrane and soluble receptors was measured as described in the text.

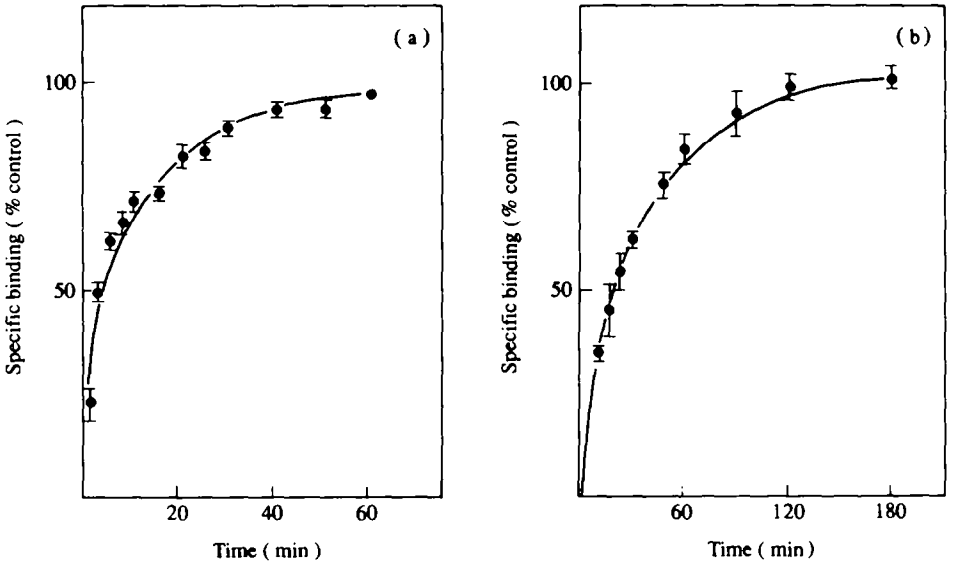

Fig. 2. Time course for association of [^{3}H]bradykinin and [^{125}I]Tyr^1.kallidin to whole NG 108-15 cells (a) and soluble binding sites prepared from NG 108-15 tumour membranes (b), respectively. Specific binding to membrane and soluble receptors was measured as described in the text.

the analogues have a lower affinity for the soluble receptor compared with the membrane located binding sites. The IC_{50} values for the soluble binding sites also correlate well with the corresponding functional assays ($R = 0.86$ and 0.85 for the NG 108-15 and uterine receptors, respectively). The similar behaviour of these analogues in the binding and functional assays for each tissue supports the view that the soluble binding activity is related to a physiological bradykinin receptor expressed by NG 108-15 cells and rat uterus.

Des-Arg^9.bradykinin is an agonist at 'B1' bradykinin receptors, and is essentially inactive at 'B2' receptors [16]. This ligand was a very poor competitor of [^{125}I]Tyr^1.kallidin binding to both soluble preparations consistent with the presence of 'B2' but not 'B1' receptors in both soluble preparations.

The effect of ACE inhibitors on [^{125}I]Tyr^1.kallidin binding to soluble receptors

It has been reported that peptidases may bind

Table 1. Comparison of IC_{50} values for bradykinin analogues in soluble and membrane binding assays from NG 108-15 cells and rat uterus, EC_{50} values for bradykinin-induced ^{45}Ca efflux assays in NG 108-15 cells and relative potencies in rat uterus contractions

Compound	IC_{50} (μM) NG cells	IC_{50} (μM) soluble NG cells	EC_{50} (μM) ^{45}Ca efflux assay	IC_{50} (μM) uterus membranes	IC_{50} (μM) soluble uterus membranes	Potency rat uterus* (Bk = 100)
Bradykinin (BK)	0.003 ± 0.0002	0.004 ± 0.003	0.002 ± 0.0002	0.0015 ± 0.0002	0.013 ± 0.0005	100
Met.Lys.BK	0.0023 ± 0.0002	0.004 ± 0.002 (2)	0.0055 ± 0.0005 (2)	0.0015 ± 0.0003	0.021 ± 0.003 (4)	100
Iodo-Tyr^0.BK	0.0015 ± 0.0002	0.0025 ± 0.002	—	0.0014 ± 0.0008	0.015 ± 0.0008 (7)	69
Lys^0.Ala^2.BK	0.017 ± 0.003	0.063 ± 0.011	0.036 ± 0.007	0.0033 ± 0.0001	0.123 ± 0.009 (4)	—
D-Phe^7.BK	0.122 ± 0.021	0.081 ± 0.012	1.99 ± 0.92	0.100 ± 0.002	0.23 ± 0.04 (4)	1
des-Arg^1.BK	2.18 ± 0.2	5.05 ± 1.9	2.5 ± 0.55	0.49 ± 0.09	7.67 ± 1.2 (4)	<0.01
des-Arg^9.BK	6.46 ± 0.6	79.3 ± 21	>21	2.53 ± 0.03 (2)	>80	0.01

The values represent the average ± SE of three separate experiments unless otherwise stated.
* Data taken from Refs 6 and 24.

bradykinin-like ligands with high affinity under conditions where their enzyme activity is inhibited [17, 18]. We found that commercial preparations of ACE were indeed able to bind [^{125}I]Tyr^1.kallidin with high affinity (K_d = 68 nM). BPP5a, is an extremely potent inhibitor of ACE binding with an $IC_{50} < 1$ nM, even in the presence of the inhibitor cocktail we have used for receptor solubilization. Therefore, we have examined the ability of BPP5a to inhibit the binding of [^{125}I]Tyr^1.kallidin to the soluble binding sites from NG 108-15 tumour membranes and rat uterus. The binding activity of both soluble receptors was unaffected by BPP5a. These results would exclude the possibility that the binding activity in these extracts is due to binding to ACE or an ACE-like enzyme.

Effect of sodium ions on [^{125}I]Tyr^1.kallidin binding to soluble receptors

The binding of tritiated bradykinin to various tissues has been shown to be modulated by both mono- and divalent cations [7, 8]. Sodium ions were the most effective monovalent ions in reducing the binding of [^{3}H]bradykinin to guinea-pig ileum membranes [7]. We have similarly found that the binding of [^{125}I]Tyr^1.kallidin to NG 108-15 tumour and rat uterine membranes is reduced by increasing concentrations of sodium ions. The reduction of binding is a consequence of a reduction in the IC_{50} value for bradykinin in the presence of sodium ions. The K_d for tumour membranes is reduced from 0.89 nM in the absence of sodium chloride to 2.4 nM in the presence of 150 mM sodium chloride. A similar shift was also seen with uterine membrane binding site. We have quantitated the specific binding of [^{125}I]Tyr^1.kallidin as a function of sodium chloride concentration for both membrane receptors (Fig. 3a and b) and obtained IC_{50} values for sodium ions of 47 and 55 mM for the NG 108-15 and uterus receptors, respectively. We have shown the corresponding soluble receptors to have very similar dependence on sodium ion concentrations (Fig. 4a and b) with IC_{50} values of 43 and 35 mM, respectively.

DISCUSSION

In this paper we report the solubilization of bradykinin binding activity from NG 108-15 tumour membranes and rat uterus. As with most receptors the binding activity is very sensitive to the environment provided by the detergent used. Both CHAPS and digitonin have been successfully used to solubilize other membrane located receptors (e.g. Refs 19–22), including the bradykinin receptor from bovine uterus [10]. However, although the properties of the binding sites in the two tissues appear to be very similar it is noteworthy that only digitonin is capable of solubilizing the binding sites from both tissues. CHAPS does not facilitate the solubilization of the bradykinin receptor with retention of binding activity from NG 108-15 tumour membranes, but will enhance the yield of receptor solubilized by digitonin from uterine tissue. It is not clear whether this difference in susceptibility to detergent of the NG 108-15 and the uterine receptors is a consequence of molecular differences in the bradykinin receptors in the two tissues, or is a result of differences in the

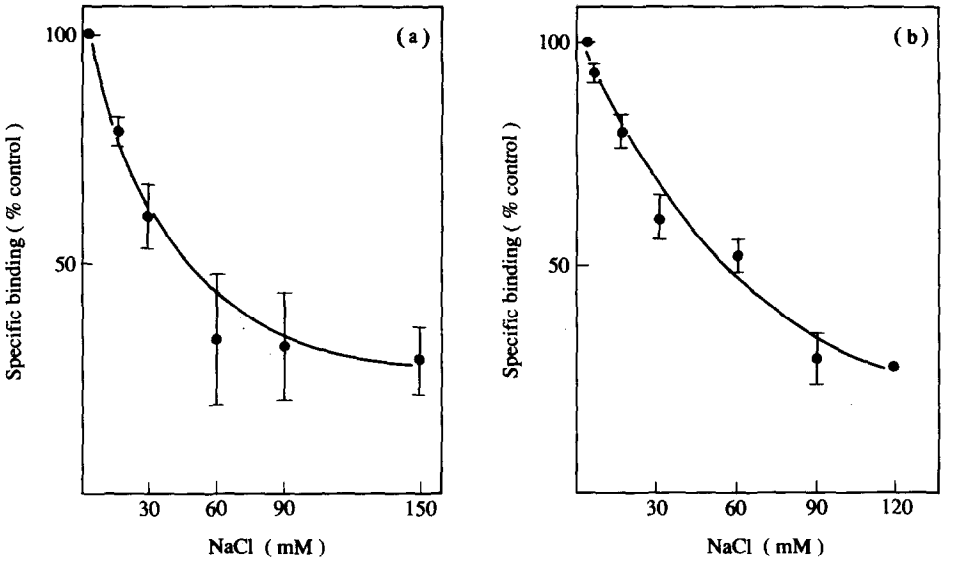

Fig. 3. Specific binding of [^{125}I]Tyr^{1}.kallidin to membranes from NG 108-15 tumours (a) and rat uterus (b) as a function of sodium chloride concentration. Specific binding to membrane preparations was measured as described in the text.

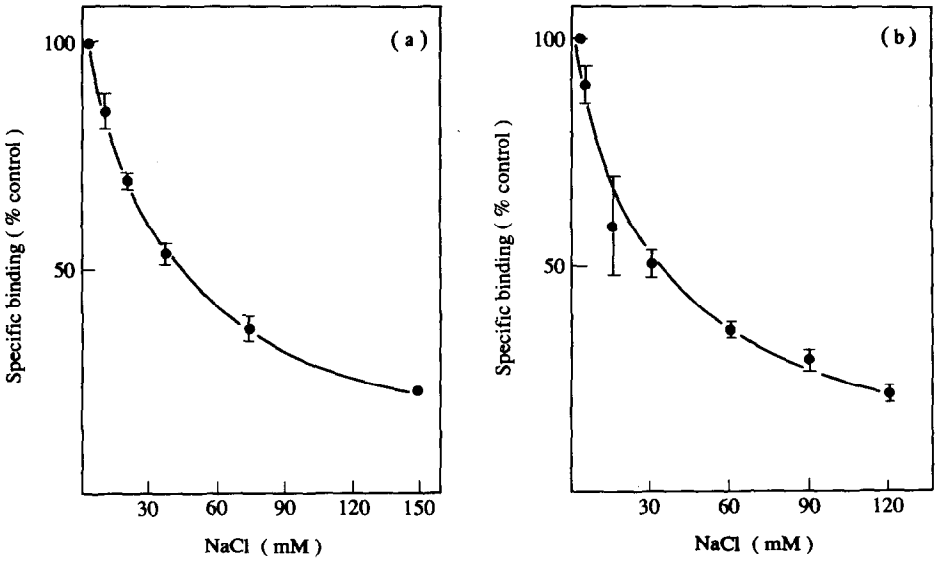

Fig. 4. Specific binding of [^{125}I]Tyr^{1}.kallidin to soluble receptor preparations from NG 108-15 tumour membranes (a) and rat uterine membranes (b) as a function of sodium chloride concentration. Specific binding to membrane preparations was measured as described in the text.

lipid composition of the two membranes and the resulting lipid/detergent/receptor complex in the solubilized form. We have tried to stabilize the soluble receptor by inclusion of various combinations of membrane lipid, however we were unable to find conditions that increased receptor stability.

The reported ability of bradykinin and analogues to bind to angiotensin converting enzyme [17] and the ability of photoaffinity probes based on kallidin to label preferentially this enzyme [23] has led us to examine the possibility that a proportion of the binding activity we have solubilized may be due to binding to ACE. We have found that ligands known to have a high affinity for the bradykinin binding sites on ACE are unable to displace binding of [^{125}I]Tyr^{1}.kallidin to either the soluble NG 108-15 or the uterine receptors, and conclude that the binding activity we have solubilized does not represent binding to ACE.

We have given careful attention to the properties of the soluble receptors to ensure that the binding activity we have solubilized truly represents the

receptor for bradykinin in the two tissues. We have therefore compared the binding properties of the membrane and soluble binding sites. For a range of bradykinin analogues there is an excellent correlation between the IC_{50} values for the soluble and membrane binding sites. In general, the IC_{50} values for the soluble binding sites are 2–10-fold higher than for the membrane receptors, probably as a direct consequence of removal of the receptor from the membrane to a detergent environment. The properties of the rat uterine receptor we have solubilized appears to be similar to those already described for the bradykinin receptor solubilized from bovine uterus for bradykinin [10, 11] despite the fact that the bovine receptor was solubilized using CHAPS alone as detergent. The IC_{50} values we have obtained for the soluble binding sites also correlate very well with the IC_{50} values in the respective functional assays. We have also shown that the affinity of bradykinin receptors on NG 108-15 tumour and rat uterine membranes is reduced by increasing sodium ion concentrations, as already described for other tissues [7, 8]. Our studies with the soluble receptors show almost identical sodium sensitivity to the corresponding membrane preparations. The results we have obtained for the soluble uterus receptor is very similar to that previously reported for the soluble bovine uterus by Frederick *et al.* [10].

We would conclude from these studies that the soluble receptors retain the same binding and sodium sensitivity as the respective membrane located receptors, and that the ligand binding properties of these binding sites correlate well with the pharmacological selectivity of the bradykinin receptors in functional assays. This evidence would support the conclusion that we have solubilized the functional bradykinin receptors present on NG 108-15 cells and the rat uterus.

The differences in susceptibility of the NG 108-15 and the uterine receptors to detergents and the differences in the kinetics of association of the membrane and soluble receptors suggest some difference in the receptors from the two tissues. However, these differences in kinetics and detergent sensitivity could just as easily be due to differences in the environment of the receptor protein in the solubilization milieu. For the limited number of agonist analogues we have studied the binding properties of the membrane receptors in the two tissues to appear very similar, both having the properties expected of the B2, but not the B1 bradykinin receptor subtype. However, unequivocal demonstration of differences in the B2 receptors in the two tissues must await more detailed pharmacological study of a range of pure antagonists in the two systems. The cloning of the bradykinin receptor would allow subsequent examination of possible biochemical differences between the receptors from the two tissue sources.

Acknowledgement—We thank Mary McNeill for her assistance with some of the calcium efflux experiments.

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