Biochemical Pharmacology, Vol. 34, No. 16, pp. 2853–2858, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

ANTI-MORPHINE ANTI-IDIOTYPIC ANTIBODIES

OPIATE RECEPTOR BINDING AND ISOLATED TISSUE RESPONSES

DAVID S. S. NG and GARY E. ISOM*

Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

(*Received* 20 *August* 1984; *accepted* 21 *January* 1985)

Abstract—Anti-idiotypic antibodies which recognize the opiate receptor were generated in guinea pigs following immunization against purified rabbit anti-morphine antibodies. The anti-idiotypic antibodies produced a concentration-dependent inhibition of [^{3}H]naloxone binding to opiate receptors in a membranous mouse brain preparation. Saturation analysis indicated that the antibodies produced a non-competitive inhibition of naloxone binding. The ability of the antibodies to interact with biological systems was investigated in *in vitro* systems. In both the isolated guinea pig ileal longitudinal muscle and mouse vas deferens, the antibodies produced a concentration-dependent, opiate agonist-like action. The anti-morphine anti-idiotypic antibodies appear to interact specifically with the opiate receptor and may serve as useful tools in characterization of this receptor system.

Anti-idiotypic antibodies, which have specificity for a variety of hormone and neurotransmitter receptors, have been produced following immunization against specific antiligand antibodies [1, 2]. Anti-idiotypic antibodies interact with the ligand binding site of the membrane bound receptor and, in several cases, can initiate a biological response following binding to the receptor. Anti-idiotypic antibodies that interact with specific receptors have been reported for the insulin [3, 4], beta-adrenergic [5, 6] and acetylcholine receptors [7, 8].

Anti-idiotypic antibodies represent a new approach to studying the functionality of receptors within their native environment. For example, Strosberg *et al.* [9] demonstrated that anti-alprenolol anti-idiotypic antibodies bind to the beta-adrenergic receptor on the surface of turkey erythrocytes; these binding sites were visualized by the use of fluorescent antibody techniques. Spontaneously generated anti-idiotypic antibodies may represent the underlying pathological mechanism of several receptor-mediated diseases. Wasserman *et al.* [7] produced the signs of myasthenia gravis following immunization of rabbits against a nicotinic cholinergic agonist. Additionally, altered insulin receptor function was produced by immunizing mice against insulin [4], and this appeared to involve an anti-idiotypic antibody response.

Recently, our laboratory reported the generation of antibodies that interact with the opiate following immunization against anti-morphine antibodies [10]. Antiserum containing anti-idiotypic antibodies inhibited specific binding of [^{3}H]naloxone to opiate receptors in mouse brain homogenates. The present study reports in more detail the binding characteristics of anti-morphine anti-idiotypic antibodies and their biological action in isolated tissues.

* Address correspondence to: Dr. Gary E. Isom, Department of Pharmacology and Toxicology, Purdue University, West Lafayette, IN 47907.

MATERIALS AND METHODS

Chemicals were obtained from the following sources: [^{3}H]naloxone (sp. act. = 50 Ci/mmole) was from New England Nuclear (Boston, MA); [^{14}C]morphine HCl (sp. act. = 58 mCi/mmole) was from Amersham (Arlington Heights, IL); methionine-enkephalin, beta-endorphin and complete Freund's adjuvant were from the Calbiochem Co. (LaJolla, CA); Trizma, 1-ethyl-3(3-dimethylaminopropryl) carbodiimide HCl, and incomplete Freund's adjuvant were from the Sigma Chemical Co. (St. Louis, MO); AH-Sepharose-4B was from the Pharmacia Fine Chemical Co. (Piscataway, NJ); 3-*O*-carboxymethylmorphine-bovine serum albumin and 3-*O*-carboxymethylmorphine, were from the Technam Co. (Park Forest, IL); levorphanol tartrate, dextrorphan tartrate and levallorphan tartrate were from Roche Laboratories (Nutley, NJ); naloxone HCl and naltrexone HCl were from Endo Laboratories (Garden City, NY); and ketocyclazocine was from Sterling Winthrop Research Institute (Rensselaer, NY).

Male Swiss–Webster mice (20–25 g) were obtained from Laboratory Supply (Indianapolis, IN), Male Hartley guinea pigs from Murphy Breeding Laboratory (Plainfield, IN), and male New Zealand White rabbits from Johnson's Bunny Ranch (Wilkinson, IN). Animals were housed in separate rooms with temperature controlled at 22° and a 12-hr alternating light–dark cycle.

Production of anti-morphine antibodies. Rabbits were immunized subcutaneously with 10 mg of carboxymethylmorphine-bovine serum albumin in 0.5 ml of phosphate-buffered saline (pH 7.4) emulsified with an equal volume of complete Freund's

adjuvant. Booster injections of 10 mg of antigen emulsified in incomplete Freund's adjuvant (1.0 ml) were given every 3 weeks after the first immunization over a period of 4 months. Seven days after the booster, the animal was bled from the inner marginal ear vein and serum was separated. To monitor the development of active immunization, the antiserum was tested for specific binding of [^{14}C]morphine by the Farr technique [11].

Anti-morphine antibodies were purified from the rabbit serum by affinity chromatography on a column containing carboxymethylmorphine bound by a carbodiimide-mediated coupling reaction to an AH-Sepharose 4B matrix [12]. A crude γ-globulin fraction of the anti-morphine antiserum was prepared from 20 ml of serum by ammonium sulfate precipitation followed by dialysis against 0.01 M (pH 7.4) phosphate-buffered saline (PBS). The γ-globulin fraction was placed in 5 ml of PBS (0.1 M, pH 6.9) and applied to the carboxymethylmorphine-Sepharose column. Following a 15 to 20-min period, the column was successively washed with 35 ml of 0.1 M PBS (pH 6.9), 20 ml of 0.1 M acetate buffer (pH 3.0), and 20 ml of 0.2 M glycine HCl buffer (pH 2.6). The specific fractions were dialyzed against 100 vol. of 0.01 M PBS (pH 7.4) containing 0.025% sodium azide for 24 hr at 4°. The [^{14}C]morphine binding capacity of each fraction was determined by the Farr assay [11] and the purified anti-morphine antibodies were stored at −20° until used. The morphine binding capacity of the acetate and glycine buffer fractions was retained following freezing over a 6-month period. For immunization of guinea pigs, the antibody fraction eluted with acetate buffer was selected since it gave the highest yield of anti-morphine antibodies.

Saturation binding of [^{14}C]morphine to anti-morphine antibodies was determined by the Farr technique [11]. Purified antibodies were incubated with various concentrations of [^{14}C]morphine (2.5 to 250 nM) overnight, and the radioactivity bound in the antigen–antibody complex was determined. In competitive inhibition studies, equal volumes (0.5 ml) of antibodies (50 μg), [^{14}C]morphine, and unlabeled inhibitor were incubated overnight followed by precipitation of bound radioactivity. Bovine gamma globulin (0.5%) in 0.01 M PBS (pH 7.2) was used as a carrier protein and diluent for the antibodies and inhibitors.

Production of anti-idiotypic antibodies. Guinea pigs were immunized against 20 μg of purified anti-morphine antibodies (the idiotype) emulsified in complete Freund's adjuvant (1.0 ml) by subcutaneous injection. Booster injections of 10 μg of antibodies emulsified in incomplete Freund's adjuvant were administered weekly.

The guinea pigs were bled weekly by cardiac puncture under anesthesia. The serum was assayed for an active immune response against anti-morphine antibodies by the interfacial (ring) test [11] and an immunoprecipitation-radioimmune assay. In the assay, 0.2 ml of 0.1 M PBS (pH 7.2) containing 200 μg of purified anti-morphine antibodies was incubated with 0.2 ml of antiserum overnight at 4° in the presence of 0.1 ml of carrier protein (bovine gamma globulin, 0.5% (w/v) in 0.01 PBS, pH 7.2). After incubation, the reaction mixture was centrifuged at 2000 *g* for 30 min. Anti-morphine antibodies remaining in the supernatant fraction were measured by the Farr technique with [^{14}C]morphine as the labeled ligand.

Opiate receptor binding assays. Opiate receptor binding was determined by a modification of the procedure of Caruso *et al.* [13]. Brains minus the cerebellum from five to ten mice were pooled and homogenized in 30 vol. (w/v) of cold Tris–HCl buffer (50 mM, pH 7.7) with a polytron PT-10 (Brinkmann Instruments, Westbury, NY) at a setting of 5 for 20 sec. The homogenate was centrifuged at 49,000 *g* for 15 min at 4°, resuspended in an equal volume of Tris–HCl buffer, and incubated alone at 37° for 30 min. Then the homogenate was recentrifuged and resuspended in Tris–NaCl buffer (50 mM Tris, 100 mM NaCl, pH 7.4 at 25°); 2.0 ml of the homogenate and either 0.1 ml of Tris–NaCl buffer or levallorphan (final concentration of 1 μM) were incubated for 10 min at 25°. Then 0.1 ml of [^{3}H]naloxone (final concentration of 0.3 to 10 nM) was added, and the incubation was continued for an additional 20 min. Bound [^{3}H]naloxone was then separated from free ligand by filtration over glass filters (Whatman GF/B) and then washed twice with 5.0 ml of cold Tris–NaCl buffer. Specific binding was calculated by subtracting the binding in the presence of 1 μM levallorphan from binding in its absence. All values were corrected to fmoles of bound naloxone/mg protein. Inhibition of naloxone binding by guinea pig antiserum was determined by assaying [^{3}H]-naloxone binding after incubation of the homogenate with antiserum at a final dilution ranging from 1:1100 to 1:22 for 60 min at 25°. Normal guinea pig serum and pre-immune guinea pig serum served as a control in the binding assays.

Isolated mouse vas deferens and guinea pig longitudinal assays. The vas deferens (both segments) were removed intact and suspended in a 45-ml tissue bath at 37° containing oxygenated magnesium-free Krebs-bicarbonate solution with 1.25 M chlorpheniramine maleate. The tissue was equilibrated for 1 hr before application of an electrical stimulation (80 V, 0.1 Hz frequency, 2 msec duration). Guinea pig anti-idiotypic serum was added directly to the bath in increasing volumes ranging from 50 to 500 μl.

The longitudinal muscle of the guinea pig ileum was isolated and mounted in oxygenated Krebs-bicarbonate solution as described for the vas deferens. One gram of tension was applied to the muscle, and the tissue was equilibrated for 1 hr before applying electrical stimulation to induce contraction. After contraction of the stimulated tissue was stabilized, aliquots of 50 μl of guinea pig antiserum were added to the bath to establish a cumulative dose–response relationship.

RESULTS

Characterization of anti-morphine antibodies. Antimorphine antibodies appeared in the serum of the rabbits within 2 weeks of the initial immunization against carboxymethylmorphine-BSA and reached a peak (10,000 pmoles morphine bound/ml serum) 10 weeks after immunization. Twenty millilitres of

Table 1. Potency of opioid ligands for displacing the specific binding of [^{14}C]morphine to purified anti-morphine antibodies

Ligand	Apparent inhibition constant (K_i) (mM)	Relative potency*
Morphine	0.019	1
Levorphan	0.253	0.039
Dextrorphan	146	0.00013
Naloxone	393	0.000048
Naltrexone	3,370	0.0000056
Ketocyclazocine	>10,000†	
Met-enkephalin	>10,000†	
Beta-endorphin	>10,000†	

* Relative potencies are expressed relative to morphine.
† Inhibition constants could not be determined due to limit of solubility in incubation mixture.

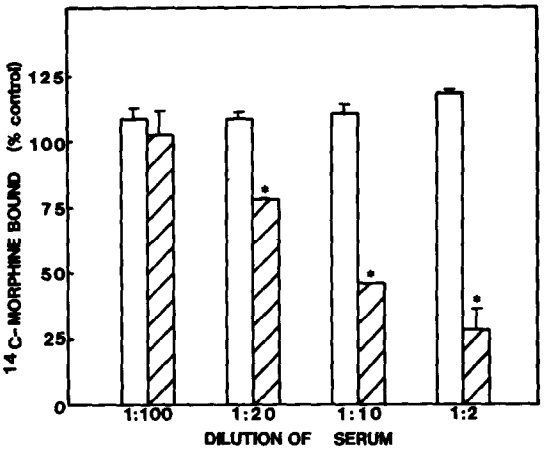

Fig. 1. Precipitation of anti-morphine antibodies by anti-morphine anti-idiotypic antiserum. Hatched bars represent guinea pig antiserum and open bars represent normal guinea pig serum. After incubation of anti-morphine antibodies with serum, residual anti-morphine antibodies in the supernatant fraction were determined using the Farr technique. Control refers to incubation of anti-morphine antibodies with phosphate-buffered saline, pH 7.2. Values are means ± S.E.M. An asterisk indicates a significant difference between antiserum and normal serum ($P < 0.05$).

rabbit anti-morphine antiserum produced approximately 4.75 mg of purified antibodies. The morphine-binding characteristics of the rabbit anti-morphine antibodies were studied by incubating the antibody with various concentrations of [^{14}C]morphine (2.5 to 250 nM). The maximal level of morphine bound was 354 pmoles/mg protein and the K_D was 19.5 nM with a Hill coefficient of 0.99. It was concluded that a homogenous population of anti-morphine antibodies was generated exhibiting one binding type which bound noncooperatively to morphine.

The ligand binding profile of the purified anti-morphine antibodies was studied by determining the ability of unlabeled opiate agonists and antagonists to compete with [^{14}C]morphine (Table 1). The antibodies were highly specific in binding the immunogen, morphine, and the related mu agonist, levorphanol. The antibodies displayed stereospecificity since levorphanol was selectively bound over its *d*-isomer, dextrorphan. Unlike the opiate receptor, antibodies did not bind opiate antagonists with high affinity since the K_i values of naloxone and naltrexone were much higher than that of morphine. The antibodies also displayed minimal cross-reactivity with opiates of other classes and, in turn, it was concluded that the binding profile of the antibodies more closely paralleled that of the mu receptor as opposed to other receptor subtypes.

Production of anti-idiotypic antibodies. Serum from guinea pigs, which were immunized against anti-morphine antibodies, was assayed weekly by the interfacial test for the ability to recognize anti-morphine antibodies. The ability of anti-idiotypic serum to immunoprecipitate anti-morphine antibodies was indicative of active immunization (Fig. 1). Animals that responded with positive interfacial tests and immunoprecipitation tests were selected for screening for anti-idiotypic antiserum in the opiate receptor binding assay.

Opiate receptor binding. The ability of the antibodies to recognize and bind the opiate receptor was tested by measuring the effect of antiserum on binding of radiolabeled opiate ligand to a membranous receptor preparation. Guinea pig antiserum produced a concentration-dependent inhibition of the specific binding of [^{3}H]naloxone (0.6 nM) to opiate binding sites. Maximal inhibition of stereospecific [^{3}H]naloxone binding varied from 37 to 71% of binding depending on the specific animal source of the polyclonal serum. Each guinea pig produced serum which displayed different degrees of interaction with the receptor. Figure 2 illustrates the

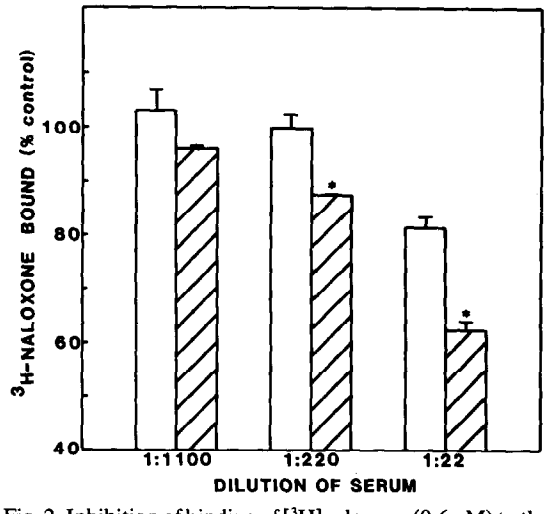

Fig. 2. Inhibition of binding of [^{3}H]naloxone (0.6 nM) to the opiate receptor by anti-morphine anti-idiotypic antiserum. Open bars represent normal guinea pig serum and hatched bars represent guinea pig antiserum. Control refers to specific binding of [^{3}H]naloxone (0.6 nM) to the opiate receptor in the absence of serum. Values are means ± S.E.M. An asterisk indicates a significant difference between antiserum and normal serum ($P < 0.05$).

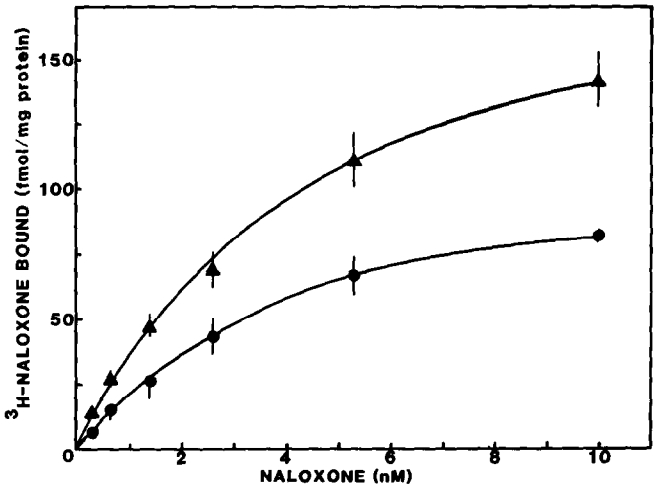

Fig. 3. Inhibition of binding of [^{3}H]naloxone to mouse brain opiate receptor by anti-morphine anti-idiotypic antiserum (1:22 dilution). Key: control (▲), and guinea pig antiserum, 1:22 final dilution (●). Values are means ± S.E.M. An asterisk indicates a significant difference from control ($P < 0.05$).

influence of various dilutions of antiserum on naloxone binding in which the maximal inhibition of binding was 37° of control at a final antiserum dilution of 1:22. To eliminate the possibility that opiates were binding to components of the antiserum, leading to inhibition of binding, a modified Farr assay was conducted with [^{3}H]naloxone and [^{14}C]morphine. Anti-idiotypic serum did not display specific opiate binding as was observed with rabbit anti-morphine serum. Although normal guinea pig serum also produced a nonspecific reduction in the binding of [^{3}H]-naloxone in the same assay (19.1% of control), the maximal inhibitory effect of guinea pig antiserum was significantly greater than that of normal guinea pig serum. Serum from an animal immunized against bovine serum albumin exhibited minimal effects on naloxone binding, paralleling that of control serum.

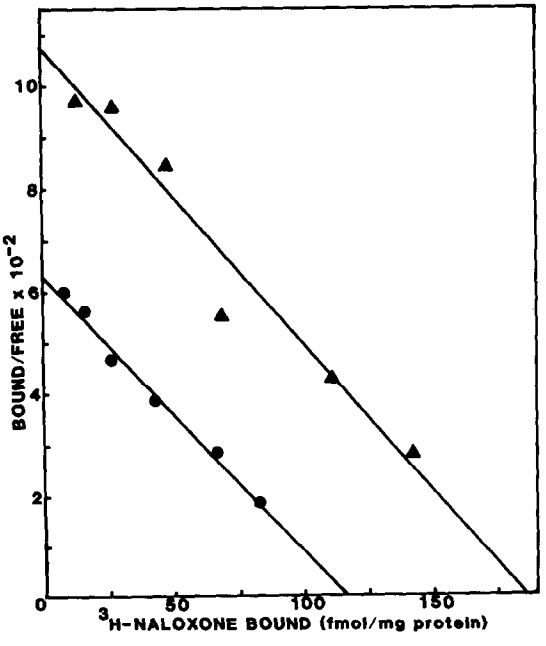

Fig. 4. Scatchard plot of [^{3}H]naloxone binding to opiate receptors in the presence of anti-morphine anti-idiotypic antiserum (1:22 dilution). Key: control (▲), and guinea pig antiserum, 1:22 final dilution (●). Results were derived from the saturation binding data of Fig. 3. The slopes were determined by linear regression analysis.

The effect of guinea pig anti-idiotypic antiserum on saturation binding of [^{3}H]naloxone to the opiate receptor was determined (Fig. 3), and Scatchard analysis of the saturation isotherm revealed that a 1:22 antiserum dilution decreased the number of opiate binding sites by 37% with a B_{max} of 116 fmoles/mg protein and a K_D of 3.87 nM (Fig. 4); the control displayed a B_{max} of 185 fmoles/mg protein and a K_D of 3.66 nM. From this study, it appeared the anti-idiotypic antiserum produced a noncompetitive inhibition of naloxone binding. It should be pointed out that normal guinea pig serum produced a decrease in binding, but the effect of normal serum was independent of the concentration added to the assay (various concentrations ranging from 1:100 to 1:22 produced identical effects on naloxone binding), whereas the anti-idiotypic serum effect was concentration dependent and could be blocked by prior incubation of antiserum with purified anti-morphine antibodies. In turn, the effects of normal guinea pig serum appeared to be nonspecific.

In vitro *responses to anti-idiotypic antiserum.* Guinea pig anti-idiotypic serum produced an opiate agonist-like inhibition of the electrically-stimulated contraction of guinea pig ileal longitudinal muscle in a concentration-dependent manner (Fig. 5). In contrast, normal guinea pig serum did not produce an alteration of muscle contraction.

In the isolated mouse vas deferens, guinea pig antiserum also inhibited the electrically-induced contractions in a concentration-dependent manner (Fig. 6). Although normal guinea pig serum produced a

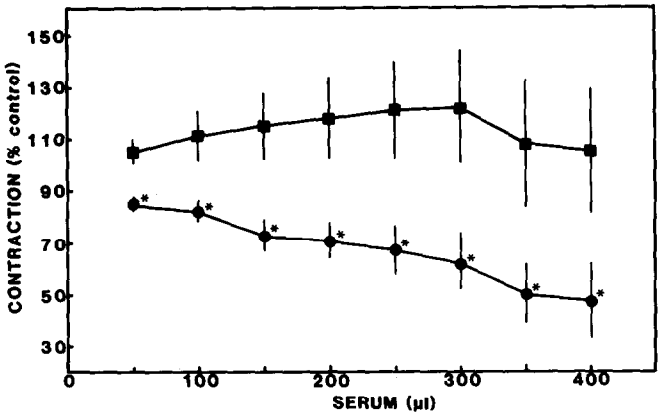

Fig. 5. Effect of anti-morphine anti-idiotypic antiserum on the contraction of guinea pig ileal longitudinal muscle. Key: (●) guinea pig antiserum, and (■) normal guinea pig serum. Control refers to contraction of tissue before addition of serum. Values are means of four experiments ± S.E.M. Asterisks indicate significant differences between the doses of normal serum and antiserum ($P < 0.05$). The curve for guinea pig antiserum is significantly different from the curve for normal guinea pig serum ($P < 0.05$).

similar effect in this tissue, the overall effect of guinea pig antiserum was significantly greater than that of normal serum. The inhibitory effect of guinea pig antiserum was greater in the guinea pig ileum since the IC_{50} in this system (350 μl) is lower than in the mouse vas deferens (500 μl).

DISCUSSION

The ability of the anti-morphine anti-idiotypic antibodies to recognize membrane opiate receptors is illustrated by their inhibition of [^{3}H]naloxone binding to opiate binding sites in mouse brain. Scatchard analysis of the data indicates that the decrease in binding was the result of a reduction of the number of binding sites without affecting the affinity of the remaining binding sites. Thus, the anti-idiotypic antibodies behaved like noncompetitive inhibitors for the brain opiate receptor in the binding assay. Inhibition of ligand binding to other receptors by anti-idiotypic antibodies has been reported. Studies on insulin receptors [3], beta-adrenergic receptors [5] and chemotactic receptors of the neutrophil [14] demonstrate inhibition of specific ligand binding to the receptor by anti-idiotypic antibodies. In the case of the beta-adrenergic receptor, anti-idiotypic antibodies exhibit either competitive antagonism [6] or noncompetitive antagonism of ligand binding [5].

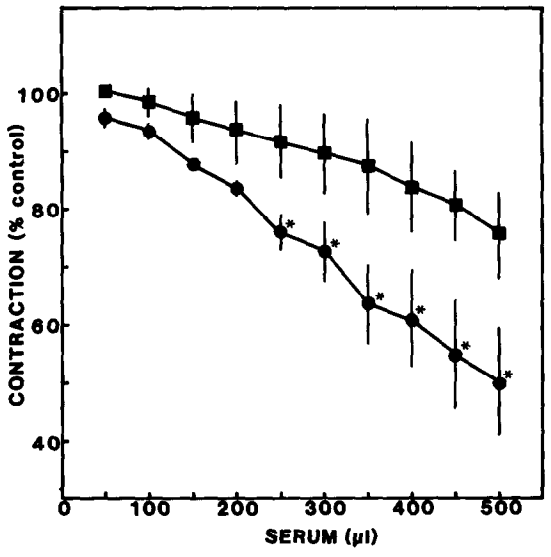

Fig. 6. Effect of anti-morphine anti-idiotypic antiserum on the contraction of mouse vas deferens. Key: (●) guinea pig antiserum, and (■) normal guinea pig serum. Control refers to contraction of tissue before addition of serum. Values are means of four different studies ± S.E.M. An asterisk indicates a significant difference between the doses of normal serum and antiserum ($P < 0.05$). The curve for guinea pig antiserum is significantly different from the curve for normal guinea pig serum ($P < 0.05$).

The anti-idiotypic response appeared to vary from animal to animal, and the serum displayed varying degrees of specificity for the opiate receptor. This may partially reflect the polyclonal nature of the serum, since serum from each animal would be composed of different clones of antibodies which recognize different epitopes at the receptor ligand binding site. The inability to produce greater than 37% inhibition may be related to the low affinity of polyclonal serum for the receptor and, importantly, only the mu opiate receptor subclass may be preferentially inhibited. The primary antibody (anti-morphine antibody) was generated against a mu agonist and retained agonist binding characteristics of the mu receptor. The mouse brain contains additional opiate receptor subclasses that may not be recognized by the anti-idiotypic serum and, in turn, naloxone would continue to exhibit binding to these subclasses. Similarly, Bidlack *et al.* [14] reported generation of monoclonal anti-antibodies directly against a partially purified opiate receptor preparation; the antibodies appeared to inhibit ligand binding to select receptor subclasses and only partially blocked ligand binding.

The anti-idiotypic antibodies not only bound to opiate receptors, but also exhibited morphine-like activity in two bioassays for opiates: the electrically

stimulated guinea pig ileal longitudinal muscle and mouse vas deferens. The guinea pig ileum was more sensitive to the inhibitory action of the antiserum. This was not unexpected since opiate receptors in guinea pig ileum and mouse vas deferens are predominately mu and delta types respectively. As a result, anti-idiotypic antibodies, which preferentially interact with mu receptors, would produce a greater response in the guinea pig ileum preparation.

The results of the present study agree with earlier findings that anti-idiotypic antibodies can bind to membrane bound receptors and initiate biological responses. Sege and Peterson [3] demonstrated that anti-idiotypic antibodies which recognize the insulin receptor alter ligand binding and exhibit biological activity. Studies on the beta-adrenergic receptors provide less conclusive results about the biological response induced by anti-idiotypic antibodies, since both stimulation and inhibition of catecholamine-mediated adenylate cyclase activity in turkey erythrocytes have been reported [5, 6]. Thus, anti-idiotypic antibodies may display both agonist and antagonist activity. In the present study, the failure of naloxone to reverse the inhibitory effect of anti-morphine anti-idiotypic antisera in both the isolated guinea pig ileum and mouse vas deferens indicates that anti-idiotypic antibodies do not function as classic opiate agonists. Either the antibodies recognize receptor epitopes outside of the active site or produce a change in receptor function which cannot be reversed by increasing ligand concentration or with opiate antagonists.

A difficulty encountered in studies with polyclonal anti-idiotypic antibodies is the low titer of antibodies generated in animals [3, 15]. In turn, a high concentration of antisera is generally required to inhibit ligand binding to receptors or to initiate a biological response. This was also observed in the present study in which a high concentration of anti-idiotypic antiserum was required to produce maximal inhibition of naloxone binding. Related to this, it is important to note that control serum, preimmune serum and serum from guinea pigs immunized against BSA produced a detectable, nonspecific effect on naloxone binding. Control serum produced a 19% inhibition of naloxone binding and, as a result, the anti-idiotypic serum evaluated in the present study could account for approximately a 20% decrease in specific naloxone binding. The cyclic nature of the anti-idiotypic response may be an additional factor that influenced the present study [7]. Recently, Strosberg [1] demonstrated that the disappearace of anti-idiotypic antibodies to beta-adrenergic receptors from the sera of immunized animals correlates with the appearance of a second set of antibodies, anti-anti-idiotypic antibodies that were generated against the anti-idiotypic antibodies. Anti-anti-idiotypic antibodies bind to both anti-idiotypic antibodies and the ligand for the receptor. This phenomenon would help explain the transient nature of the anti-idiotypic response and suppression of the biological effect induced by anti-idiotypic antibodies [3, 5, 7]. Present studies are being directed towards production of monoclonal anti-idiotypic antibodies, which may overcome many of the problems associated with polyclonal antibodies.

Acknowledgements—This research was supported by Research Grant NS 19584 from the National Institutes of Health. The authors would like to thank Ms. Della Edel for technical assistance in completion of this study.

REFERENCES

1. A. D. Strosberg, *Springer Semin. Immunopath.* **6**, 67 (1983).
2. J. C. Venter, *Surv. immun. Res.* **2**, 302 (1983).
3. K. Sege and P. A. Peterson, *Proc. natn. Acad. Sci. U.S.A.* **75**, 2443 (1978).
4. Y. Shecter, R. Maron, D. Elias and I. R. Cohen, *Science* **216**, 542 (1982).
5. A. B. Schreiber, P. O. Couraud, C. Andre, B. Vray and A. D. Strosberg, *Proc. natn. Acad. Sci. U.S.A.* **77**, 7385 (1980).
6. C. J. Homey, S. G. Rockson and E. Haber, *J. clin. Invest.* **69**, 1147 (1982).
7. N. H. Wasserman, A. S. Penn, P. I. Freimath, N. Treptow, S. Wentzel, W. L. Cleveland and B. F. Erlander, *Proc. natn. Acad. Sci. U.S.A.* **79**, 4810 (1982).
8. W. L. Cleveland, N. H. Wasserman, R. Sarangarajan, A. S. Penn and B. F. Erlander, *Nature, Lond.* **305**, 56 (1983).
9. A. D. Strosberg, P. O. Covrand, O. Durieu-Trautmann and C. D. Delavier-Klutchko, *Trends pharmac. Sci.* **3**, 282 (1982).
10. D. S. S. Ng and G. E. Isom, *Eur. J. Pharmac.* **102**, 187 (1984).
11. J. S. Gravey, N. E. Cremer and D. H. Sussdorf, *Methods in Immunology*, p. 301. W. A. Benjamin, Reading, MA (1977).
12. F. A. Wahid and G. E. Isom, *Res. Commun. Subst. Abuse* **1**, 451 (1980).
13. T. P. Caruso, D. L. Larson, P. S. Portoghese and A. E. Takemori, *J. Pharmac. exp. Ther.* **213**, 539 (1980).
14. J. M. Bidlack, R. R. Denton and L. W. Harwell, *Life Sci.* **33** (Suppl. I), 151 (1983).
15. W. A. Marasco and E. L. Becker, *J. Immun.* **128**, 963 (1982).