the Tyr^2 aromatic group cannot be over the tocin ring. We assume that the receptor is also flexible and can adopt two conformations. In the absence of hormone, it adopts a conformation that binds the oxytocin conformers with right-handed disulfide chirality. This inactive state is then stabilized by rigid right-handed disulfide containing analogues such as 1-penicillamine derivatives that act as antagonists (*24*). In contrast, strong agonists stabilize the receptor in a conformation complementary to the left-handed disulfide oxytocin conformer. An efficient transduction of the biological response would then depend on both a low energy barrier between conformers as in deamino-oxytocin and a strong interaction with the receptor in the active form.

Our model predicts that agonist activity will be enhanced either by kinetic effects involving flexibility and interconvertibility of the two conformers or thermodynamic effects that stabilize the left-handed conformer or increase its interactions with the receptor. Modifications at side chains (for example $Phe^2 \rightarrow Tyr^2$ and $Gln^4 \rightarrow Thr^4$) that increase potency (*6, 25*), may often be due to optimization of direct interactions with the receptor—in this case formation of hydrogen bonds. However, substitutions at the same positions may also affect flexibility and have kinetic effects. Thus, replacement of Gln^4 by glycine may result in a decrease of activity due to loss of a direct interaction with the side chain but a compensatory increase of potency due to increased flexibility; there is evidence that the existence of Gly^4 leads to rearrangements in the tocin ring similar to those in deamino-oxytocin.

In summary, x-ray analysis of crystal structures of deamino-oxytocin emphasizes the importance of intramolecular hydrogen bonds which appear to be retained in solution and probably at the receptor. They also highlight the conformational flexibility of this peptide hormone and define conformers that may play a role in receptor binding and biological activation.

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Circulating Atrial Natriuretic Peptides in Conscious Rats: Regulation of Release by Multiple Factors

ROBERT ESKAY, ZOFIA ZUKOWSKA-GROJEC, MARKUS HAASS, JITENDRA R. DAVE, NADAV ZAMIR

Cardiocytes in the atria contain a prohormone that gives rise to atrial natriuretic peptides (ANP's), which have intrinsic hemodynamic regulatory activity. The distribution of ANP's in the brain suggests the involvement of these peptides in central cardiovascular regulation. In conscious rats with chronic indwelling catheters, volume loading with isotonic saline or glucose increased the amount of circulating immunoreactive ANP's by a factor of 4 to 5, as determined by radioimmunoassay. Hyperosmotic challenge with a hypertonic NaCl solution or anesthesia with halothane caused similar increases in plasma ANP's. Results obtained with the denervated-heart preparation indicate that neuronal influences are important in the release of ANP's induced by volume loading. As judged from reversed-phase high-performance liquid chromatography of extracted plasma and radioimmunoassay of collected fractions, the circulating physiologically important ANP's in the conscious rodent appear to be α-rANP(5–28) (atriopeptin III) and either α-rANP(3–28) [ANF(8–33)] or α-rANP(1–28) (ANF).

FOR SEVERAL DECADES, THE MAMMALIAN cardiac atria have been known to have a role in blood volume homeostasis (*1*), and more recently atrial cardiocytes have been shown to contain secretory storage granules that are characteristic of endocrine or peptide secretory cells (*2*). Intravenous infusion of atrial extracts revealed potent natriuretic, diuretic, and vasorelaxant substances (*3*) termed atrial natriuretic factors. More recent investigations have shown that atrial tissue contains a number of biologically active peptides (*4*) collectively termed atrial natriuretic peptides (ANP's). Cardiac ANP's have been isolated, synthesized, and shown to have intrinsic hemodynamic regulatory activity (*5*). The amino acid sequence of the preprohormone in the atria has been deduced from sequence analysis of the DNA in several mammalian species; the rat preprohormone consists of 152 amino acids (*6*). Multiple ANP's (*6*), which constitute the carboxyl terminus of the precursor, have been isolated from rat atria. They vary in length from 21 to 126 amino acids. Each peptide contains a core sequence with a disulfide bridge, which is required for biological activity. Some of the substances isolated from the atria and thought to be ANP's, could be purification artifacts. Therefore, it is not yet certain which of the circulating ANP's obtained from conscious animals are physiologically important.

Although the primary sources of ANP's are the cardiac atria, immunoreactive ANP's

R. Eskay, Section of Neurochemistry, Laboratory of Clinical Studies, Division of Intramural, Clinical and Biological Research, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892.
Z. Zukowska-Grojec, Department of Physiology and Biophysics, Georgetown University School of Medicine, Washington, DC 20007.
M. Haass, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.
J. R. Dave, Section of Neurochemistry, Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892.
N. Zamir, Hypertension-Endocrine Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

(irANP's) are present throughout the central nervous system. Our immunocytochemical findings (*7*) and those of others (*8*) indicate that ANP-containing cell bodies are present primarily in the preoptic-hypothalamic areas, but also in the amygdala, mesencephalon, and pons. The largest collection of ANP-containing cell bodies in the brain was observed in nuclei of the anteroventral third ventricle region. This region is involved in the development and maintenance of experimental hypertension and in fluid and electrolyte balance (*9*). ANP-containing fibers are present in the vicinity of cell bodies as well as in the external zone of the median eminence, posterior pituitary gland, and spinal cord (*7*). Radioimmunoassay (RIA) findings of the distribution of ANP's in the central nervous system are consistent with results obtained in immunocytochemical localization studies (*10*). The distribution of brain ANP's suggests they participate in central cardiovascular regulatory events and have the potential to monitor extracellular fluid volume and electrolyte levels via the anteroventral third ventricle region and possibly to influence the release of certain pituitary gland hormones (*11*).

Using established methodology (*12*), we generated an antiserum to α-rat ANP(5–28) (ATP III). The characteristics of this antiserum in routine RIA reveal a maximum sensitivity of 1 pg per tube, with an intra-assay sample variation of 3 percent and an interassay sample variation of 5 percent (*10*).

Since changes in systemic electrolytes and volume expansion are possible modulators of ANP release, we examined the effect of short-term volume loading and hyperosmotic stimuli on the amounts of circulating ANP's. Indwelling catheters were placed in the femoral vein and artery of adult male rats (Sprague-Dawley, 325 to 375 g) 24 hours before experimental manipulation. Catheterized animals received an infusion via the femoral vein of either isotonic saline (20 ml per kilogram of body weight), 5 percent glucose (20 ml/kg), or hypertonic saline (1 ml containing 2.8 meq of NaCl per kilogram) for 1 minute. Since preliminary results revealed that peak circulating levels of irANP occurred between 1 and 2 minutes after infusion, 1.5 ml of blood was withdrawn from the femoral artery at approximately 1.5 minutes and placed immediately in chilled tubes containing 2.25 mg of EDTA and 1.5 trypsin-inhibitor units of aprotinin. Cellular elements were separated by centrifugation at 4°C, and plasma was decanted into tubes that had been chilled in dry ice. Each plasma sample was stored at −60°C until it was assayed. Plasma samples were thawed, and irANP's were extracted from plasma for determination of irANP levels (*13*).

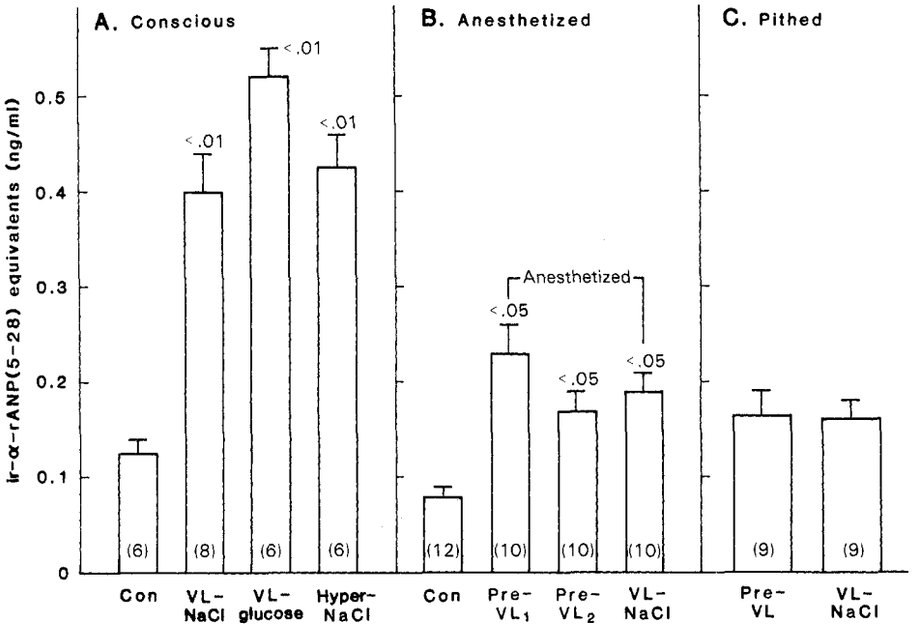

Fig. 1. Effect of various manipulations on plasma levels of immunoreactive ANP's in conscious, anesthetized, or pithed rats. Abbreviations are as follows: Con, control; VL, volume loading; Hyper, hyperosmotic; and Pre, preceding indicated treatment. Columns with vertical bars represent the mean plus SEM for each treatment. The number of animals for each treatment is given in parentheses and numbers above vertical bars represent *P* values compared to controls with the protected least-significant difference method (*22*).

Conscious, unrestrained control animals with indwelling catheters had irANP levels of 125 ± 15.6 (SEM) (Fig. 1A) or 80 ± 10.2 pg/ml (Fig. 1B) in two independent experiments. Basal irANP levels in these conscious rats are similar to those reported by Horky *et al.* (*14*). Surgical stress and anesthetics, with the exception of pentobarbital (*14, 15*), appear to increase basal irANP levels five- to 15-fold (*14, 16*). Volume loading with either 5 percent glucose or 0.9 percent saline increased plasma levels of irANP four- to fivefold (Fig. 1A). Hyperosmotic challenge (Fig. 1A) also resulted in a rapid increase in plasma ANP's, similar to that observed after volume loading. This suggests that ANP-containing cardiocytes may respond directly to changes in systemic electrolytes such as sodium or chloride ions. In contrast, basal irANP's were not significantly altered after a 1-ml infusion of 0.9 percent saline.

In conscious animals, mechanical (25 percent blood volume expansion) or hyperosmotic challenge induced a rapid release of ANP's. However, since hyperosmotic challenge increases plasma osmolarity by 13 percent and rapidly shifts fluid into the vascular space, the involvement of the atrial stretch response cannot be ruled out. In an attempt to clarify the role of a neuronal reflex versus a direct cardiac effect as an explanation for observed volume loading–induced release of ANP's, we anesthetized groups of animals with halothane and subjected them either to bilateral vagotomy or sham vagotomy and then to volume loading. Exposure to halothane for 15 minutes resulted in a threefold increase in plasma irANP's (Fig. 1B, con versus pre-VL_1). In the same experiment, 25 minutes after the onset of anesthesia, animals were vagotomized or subjected to sham operation; a third blood sample (pre-VL_2) was taken at 30 minutes after onset of anesthesia. Anesthesia was continued for an additional 10 minutes at which time each volume loading with 0.9 percent NaCl was begun; a final blood sample was taken 1.5 minutes later. Because vagotomy in halothane-anesthetized rats did not alter circulating ANP levels or modify release of ANP's induced by volume loading, pre-VL_2 samples and the final samples (VL-NaCl) were pooled for statistical analysis of ANP's from animals with sham operations and those with vagotomies (Fig. 1B). Halothane anesthesia increased basal irANP levels throughout the duration of anesthesia and blocked the release of ANP's induced by volume loading. Since the site of action of volume loading–induced release of ANP's is not known, whether or not the blocking action of halothane occurs directly at the myocardial level or indirectly through alterations of neuronal input to the heart is not clear. Pentobarbital anesthesia, in contrast to halothane anesthesia, was reported not to block volume load-

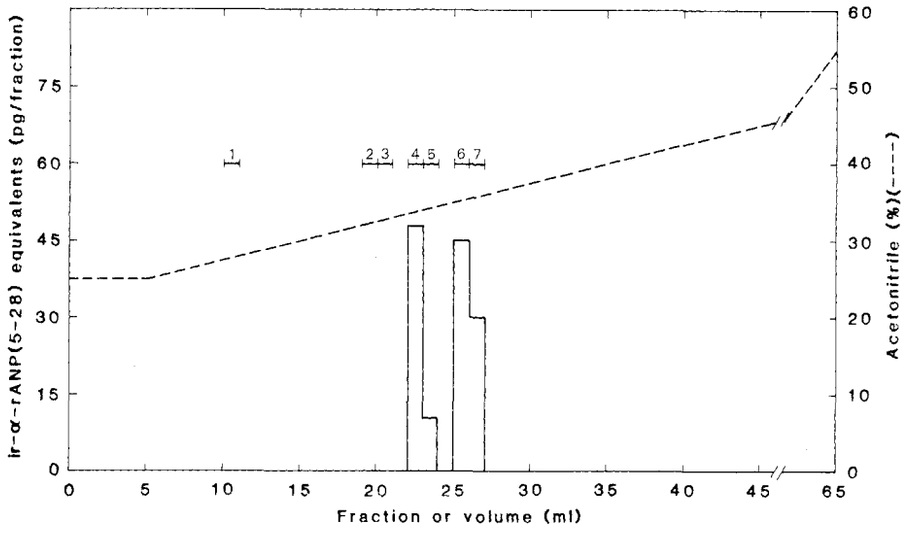

Fig. 2. Characteristic HPLC elution profile of various synthetic ANP's and immunoreactive ANP's in plasma of conscious rats. Open columns indicate the elution position of irANP's in extracted plasma. The numbered horizontal bars indicate the primary elution fraction of the following synthetic ANP's: (1) α-rANP(5–25), atriopeptin I; (2) α-rANP(5–27), atriopeptin II; (3) α-rANP(4–27), auriculin A; (4) α-rANP(5–28), atriopeptin III; (5) α-rANP(4–28), auriculin B; (6) α-rANP(3–28), ANF(8–33); and (7) α-rANP(1–28), ANF.

ing–induced release of ANP's (*15*). Although changes in circulating ANP levels induced by volume loading in pentobarbital-treated animals parallel our observed changes in conscious animals, caution should be exercised in the interpretation of results obtained in anesthetized animals because of the possibility of the differential effects of anesthetics on myocardial and neuronal tissue.

Complete cardiac denervation in the pithed rat preparation (*17*), which removes both humoral influences of central nervous system origin and direct neuronal control of the heart via the vagal and sympathetic nerves, blocked the volume loading–induced release of ANP's (Fig. 1C, pre-VL versus VL-NaCl). The results obtained with the denervated heart preparation suggest that neuronal influences are important in the phenomenon of volume loading–induced release of ANP's from the atria.

In an attempt to correlate cardiovascular parameters with the release of ANP's, we monitored mean arterial blood pressure and heart rate continuously throughout each experiment (*18*). In diverse preparations (Fig. 1), alterations in heart rate and mean arterial blood pressure were not correlated in any consistent directional manner with changes in ANP release; therefore, monitoring of these parameters offers no predictive information that relates to ANP release.

Although several ANP's have been isolated from cardiac atria, which of the atrial storage forms are released in conscious animals under physiological conditions is unknown. Extraction (*13*) of irANP's from plasma obtained from conscious animals, separation of ANP's by reversed-phase high-performance liquid chromatography (HPLC) (*19*), and quantification by RIA revealed the presence of two major peaks of immunoreactivity (Fig. 2). Comparison of the elution profile of plasma ANP's obtained from conscious animals with various synthetic ANP's suggests that the major circulating forms of ANP's are α-rANP(5–28) (atriopeptin III) and either α-rANP(3–28) [ANF(8–33)] or α-rANP(1–28) (ANF). Confirmation that the primary circulating ANP's in the conscious rat are α-rANP(5–28) and either α-rANP(3–28) or α-rANP(1–28) was achieved when quantification of HPLC fractions with an antiserum (antiserum to α-human atrial natriuretic factor; Peninsula RAS 8798) revealed an HPLC elution profile of irANP's identical to that observed with our antiserum. Unequivocal identification of plasma ANP's is not possible through the combined use of HPLC and RIA; however, recent investigations have indicated through HPLC and peptide sequence analysis (*20*) that the primary circulating ANP is α-rANP(1–28), with substantially smaller amounts of α-rANP(5–28). In our study the ratio of α-rANP(5–28) to our presumed α-rANP(1–28) (second HPLC peak) is different from that obtained by others (*20*). This discrepancy may reflect alterations in the forms of ANP released in vivo under different experimental conditions.

An understanding of the regulation of the release of ANP's from the atria is in the developmental stage, and it appears that basal irANP plasma levels in the rat range from 80 to 100 pg/ml. Because there are minor variations in HPLC runs, our data are not inconsistent with the idea that α-rANP(1–28) is the primary circulating form of ANP in the rat. Volume loading and hyperosmotic challenge increased the release of cardiac ANP's in the conscious rat. Additional experiments are needed in the conscious animal to clarify the suggested modulatory role of cholinergic, adrenergic, or peptidergic substances (*21*) on the release of ANP's from the cardiac atria.

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12. T. E. Adrian and D. J. O'Shaughnessy, in *Radioimmunoassay of Gut Regulatory Peptides*, S. R. Bloom and R. G. Long, Eds. (Praeger, New York, 1982). Male New Zealand White rabbits were immunized with α-rANP(5–28) coupled to bovine thyroglobulin by carbodiimide. On the basis of cross-reactivity studies, our antiserum appears to be both conformational and midportion-directed. It recognizes various ANP's as follows: α-rANP(1–28) (ANF), α-rANP(3–28) [ANF(8–33)], α-rANP(4–28) (auriculin B), α-rANP(5–28) (ATP III), α-rANP(5–27) (ATP II), and α-rANP(5–25) (ATP I) on an equimolar basis. In contrast it binds to α-rANP(4–27) (auriculin A) with 2.5 times greater avidity than to ATP III, and it recognizes α-human ANP(1–28)

less than 0.25% as well as it recognizes rATP III. In the original description of our antiserum (*10*), we erroneously reported, because of a bad lot of synthetic α-rANP(1–28) obtained from Peninsula Labs, that our antiserum recognized α-rANP(1–28) only 10% as well as it recognizes α-rANP(5–28).
13. Disposable syringes were placed on C_{18} Sep-Pak cartridges that were activated by passing 25 ml of HPLC-grade methanol and washed by passing 20 ml of HPLC-grade distilled water. Frozen plasma samples were thawed and rapidly portioned (1 part) into the syringes, and 4 parts of ice-cold 4% acetic acid were added immediately afterward. The acidified plasma samples were pushed through the Sep-Paks at a flow rate of approximately 2 ml/min. Each sample-loaded Sep-Pak was washed with 10 ml of 4% acetic acid solution and samples were eluted within 30 seconds with 4 ml of a solution consisting of 3 parts of acetonitrile and 1 part of 4% acetic acid. The effluent was collected and evaporated to dryness in a Speed Vac concentrator. Portions of the samples were either suspended in buffer for RIA or in 10% acetonitrile containing 0.1% trifluoroacetic acid for HPLC. The recovery of synthetic ATP III, ANF(8–33), or ANF, when added to rat plasma containing EDTA and aprotinin, and extracted as outlined, ranged from 89% to 94%. There were no statistically significant differences in the recovery of the individual peptides.
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18. In conscious animals volume loading did not alter basal values of mean arterial blood pressure (105 ± 1.1 mmHg, mean ± SEM) or heart rate (346 ± 7.7 beat/min) throughout the experiment. In contrast, hyperosmotic challenge in conscious animals resulted in a substantial increase of mean arterial pressure at 2 minutes (128 ± 3.6 mmHg) and 10 minutes (139 ± 3.1) after the onset of the infusion, whereas an increase in heart rate observed at 2 minutes (455 ± 8) had subsided to basal levels by 10 minutes. The basal value of mean arterial pressure was slightly lower in halothane-anesthetized animals (96 ± 2.0 mmHg) than in conscious animals and declined to 73 ± 2.9 mmHg 2 minutes after volume loading, whereas the heart rate response was similar to that of conscious rats. Cardiovascular parameters in the pithed rat were quite different from those of other groups; mean arterial pressure and heart rate, which were 64 ± 5.0 mmHg and 386 ± 19 beat/min before volume loading, were 96 ± 7.5 and 449 ± 18, respectively, 2 minutes after volume loading.
19. A plasma extract obtained from conscious rats was subjected to HPLC with a Gilson dual-pump system (pump A, 0.1% trifluoroacetic acid in water; pump B, 0.1% trifluoroacetic acid in acetonitrile) and an Altex ultrasphere-ODS column (4.6 × 250 mm; 5 μm particle size). Acetonitrile (25%) was maintained for 5 minutes after injection of the plasma extract; a linear gradient from 25% to 55% acetonitrile was then maintained for 60 minutes. Fractions (1 ml) were collected, dried, and subjected to RIA (Fig. 2). Synthetic ANP's were chromatographed under identical conditions and their elution profile is as indicated in Fig. 2.
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Protection of Cattle Against Foot-and-Mouth Disease by a Synthetic Peptide

RICHARD DIMARCHI, GERALD BROOKE, CHARLES GALE, VICTOR CRACKNELL, TIMOTHY DOEL, NOEL MOWAT

A chemically synthesized peptide consisting essentially of two separate regions (residues 141 to 158 and 200 to 213) of a virus coat protein (VP1) from the 0_1 Kaufbeuren strain of foot-and-mouth disease virus was prepared free of any carrier protein. It elicited high levels of neutralizing antibody and protected cattle against intradermolingual challenge by inoculation with infectious virus. Comparative evaluation of this peptide with a single-site peptide (residues 141 to 158) in guinea pigs suggests the importance of the VP1 carboxyl terminal residues in enhancing the protective response.

FOOT-AND-MOUTH DISEASE (FMD) IS a major, although rarely fatal, disease of domesticated animals; an outbreak of the disease in cattle can be financially devastating. In countries where the disease is endemic, the commonly used method of control is vaccination with an inactivated virus preparation. Approximately 10^9 doses of vaccine are administered annually in South America alone (*1*). The use of a synthetic protein–peptide vaccine for FMD has attracted much attention (*2, 3*) since it offers the advantage of a highly pure, defined, and safe alternative to the conventional vaccine.

In early work, isolated VP1 coat protein from the FMD virus (FMDV) was able to induce neutralizing antibody (*4, 5*) and protective immune (*5*) responses in swine and cattle (*6*). These results were later confirmed when cattle and swine were protected against FMDV serotype A challenge after vaccination with a biosynthetic VP1 fusion protein (*2, 7*). Strohmaier *et al.* (*8*), on the basis of a series of chemical and enzymatic degradations of VP1 from the 0_1 Kaufbeuren (0_1K) strain of FMDV, suggested that the important epitopes were centered in two small regions. These two sites are represented by residues 146 to 154 and 201 to 213. The studies of Bittle *et al.* (*9*) revealed a particularly active VP1 peptide of residues 141 to 160 which, when coupled to keyhole limpet hemocyanin (KLH), provided guinea pigs a neutralizing antibody response superior to that provided by VP1 protein. However, the successful extension of these guinea pig results to cattle, the primary target animal, by this conjugate or an analogous substance has yet to be reported (*10*). Furthermore, the presence of KLH or any other carrier as a required ingredient detracts from the advantages of peptide vaccines. The contribution of carrier selection, purity, and conjugate molecular heterogeneity (as introduced by chemical construction) to the final results can make identification of the active entity difficult.

We considered the possibility of a single-peptide vaccine consisting essentially of the two previously identified immunogenic regions connected by a diproline spacer and terminating on each end with cysteine residues. Both peptidyl regions would be included to increase the molecular size while providing a second site of some importance relative to residues 141 to 160 (*11*). The diproline spacer was inserted as a means of increasing the likelihood of interaction between the two sites through a presumed induction of a secondary structural turn. The final aspect of the synthesis was the placement of cysteine residues at or near each terminus for the purpose of polymerization as a possible means of eliminating the necessity of a carrier molecule. The following peptides, corresponding to the sequence in VP1 of the 0_1K serotype, were prepared by solid-phase synthetic methodology (*12*): 141–158-ProCysGly (21-residue peptide), 200–213-ProProSer-141–158-ProCysGly (38-residue peptide), and CysCys-200–213-ProProSer-141–158-ProCysGly (40-residue peptide). The first of

R. DiMarchi and G. Brooke, Eli Lilly Research Laboratories, Indianapolis, IN 46285.
C. Gale, Eli Lilly Research Laboratories, Greenfield, IN 46140.
V. Cracknell, Eli Lilly Research Laboratories, Windlesham, Surrey, United Kingdom.
T. Doel and N. Mowat, Animal Virus Research Institute, Pirbright, Surrey, United Kingdom.