ent mode of transmission, and a unique terminal genome sequence define a new and separate genus of Bunyaviridae. We propose the name *Hantavirus* for this genus.

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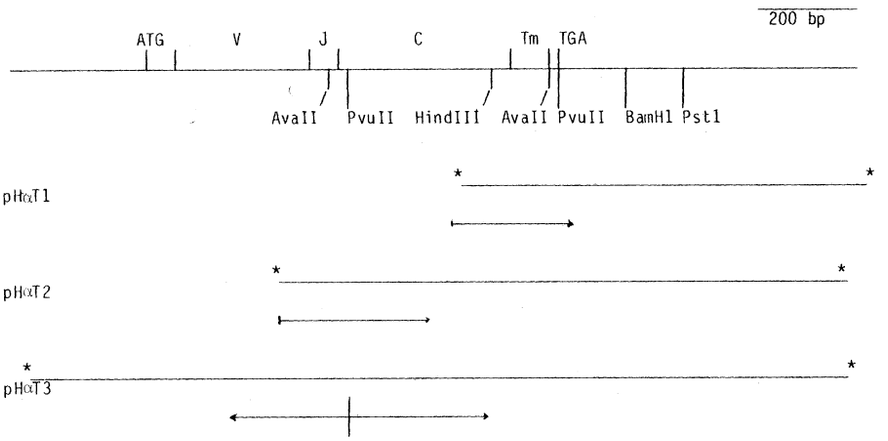
# Gene for α-Chain of Human T-Cell Receptor: Location on Chromosome 14 Region Involved in T-Cell Neoplasms

Abstract. *A human complementary DNA clone specific for the α-chain of the T-cell receptor and a panel of rodent × human somatic cell hybrids were used to map the α-chain gene to human chromosome 14 in a region proximal to the immunoglobulin heavy chain locus. Analysis by means of in situ hybridization of human metaphase chromosomes served to further localize the α-chain gene to region 14q11–q12, which is consistently involved in translocations and inversions detectable in human T-cell leukemias and lymphomas. Thus, the locus for the α-chain T-cell receptor may participate in oncogene activation in T-cell tumors.*

The T-cell antigen receptor is a 80,000 to 90,000 dalton heterodimeric glycoprotein which, in reducing conditions, yields two subunits of 40 to 50 kD each (*1*). Clones of complementary DNA (cDNA) encoding the β-chain of the T-cell receptor have been isolated from murine (*2*) and human (*3*) T lymphocyte cDNA libraries. More recently, cDNA clones encoding what is believed to be the α-




```
                20                  40                  60
gGtgcaacTc aCTACTgctg tcCACCaata CTcTTCTGGT ATGTgCAaTA CCCcaacCAa GGaCTcCAGC

        80                  100                 120                 140
TtCTCCTgaA GTACacaTCa GcgGcCaCcc TGGTTaAAGG caTcAAcGGt TTTGAGGCTG AaTTtAagAA

                160                 180                 200
GAGtgAaaCc TCCTTCCACC TGacGAAAcC CgCaGccCAt atGAGCGACg CGGCTGaGTA CTTCTGTGCT
                    V->   <-J
        220             | 240                 260                 280
GTGAGtGatc TcGaAccgaa caGCAGTGct tcCAAGaTaA tcTTTGGATC aGGgACcAgA cTCAgcaTcC
J>  <-C
 |              300                 320                 340
ggCCAaAtAT CCAGAACCCt GAcCCTGCcG TGTACCAGcT gAgAGActCT aaaTCcagtG ACAagtCtgT

        360                 380                 400                 420
CTGCCTaTTC ACCGAtTTTG AtTCtCAAAc aAAtGTGtCa cAAAgtAaGG AtTCTGatgt GTatATCACa

                440                 460                 480
GACAAAACTG TGCTGGACAT GAggtCTATG GAcTtCAAGA GCAAcaGtGC tgTgGCCTGG AGCAACaAaA

        500                 520                 540                 560
CtAGCTTtgC atgtgCAaac gccttcaaca AcAgCaTtAt tccagaagAC aCCttCTtCC CCAGcccAGA

                580                 600                 620
aaTTtCCTGT GATGtCAaGT TggtCGAGAA AAGCTTTGAA ACAGATcgGA ACCTAAACTT TCaAAACCTG

C-><Tm 640                  660                 680                 700
TCAGTgATtG GgtTCCGAAT CCTCCTcCTG AAAGTgGCgG GgTTTAAtcT GCTCATgACG tTGcGGCTGT

                720
GGTCCAGcTG AGactTGCAA GttgtAag
```

Fig. 1. (Top) Restriction map and strategy used to determine the nucleotide sequence of cDNA clones pH T1, pH T2, and pH T3. Arrows indicate the directions and the extent of a nucleotide sequence determination; (*) indicates artificial Pst I sites generated by the tailing process. (Bottom) Partial nucleotide sequence of the cDNA clone of the human T-cell receptor of Jurkat T cells. The leader, V, J, C transmembrane (Tm), and cytoplasmic regions are indicated. Regions of homology to the mouse cDNA α-chain clones described by Chien *et al.* (*4*) and by Saito *et al.* (*5*) are also indicated by capital characters. A cDNA library was prepared from 0.5 μg of polyadenylate [poly(A)]-containing RNA isolated from Jurkat (clone E) human T cells (*9*) by the method of Gubler and Hoffman (*20*) with minor modifications. The C-tailed double-stranded cDNA was annealed to the G-tailed Pst I site of PUC 8 (*21*). JM109 cells were transformed by the method of Hanahan (*22*) and approximately 700,000 colonies were collected. The cDNA library was screened by the method of Grunstein and Hofner (*23*) using the mouse α-chain-specific cDNA clone TT11. Thirty-six positive clones were isolated. Three clones (pHαT1, pHαT2, and pHαT3) were analyzed in detail and shown to be 900, 1200, and 1600 bp in length. Restriction map analysis and partial overlapping sequences of the three clones indicated that they were homologous. Sequencing was carried out in M13, mp18, and mp19 by the Sanger dideoxy method (*24*).

chain have been isolated from murine T lymphocyte cDNA libraries (*4–5*). The β-chain consists of four separately encoded regions, that is, the variable (V), diversity (D), joining (J) and constant (C) regions; and the α-chain consists of at least the V, J, and C regions (*2–4*). All of these regions are rearranged during T-cell differentiation by mechanisms that probably resemble those operative for the immunoglobulin genes during B-cell differentiation (*6*).

The gene encoding the β-chain of the T-cell receptor has been mapped to chromosome 6 in the mouse and to chromosome 7 in man (*7*). However, unlike the chromosome carrying the immunoglobulin genes, which are frequently involved in a variety of B-cell lymphomas and leukemias (*8*), the chromosome region to which the T-cell receptor β-chain gene has been mapped in both man and the mouse does not seem to be involved at any significant frequency in T-cell malignancies. Thus, it was of interest to identify the human chromosome carrying the gene for the α-chain of the T-cell receptor to determine whether rearrangements of this gene play a role in the pathogenesis of T-cell lymphomas and leukemias. We report here that indeed the gene encoding the α-chain of the human T-cell receptor maps to the chromosomal region that is consistently involved in rearrangements in T-cell malignancies.

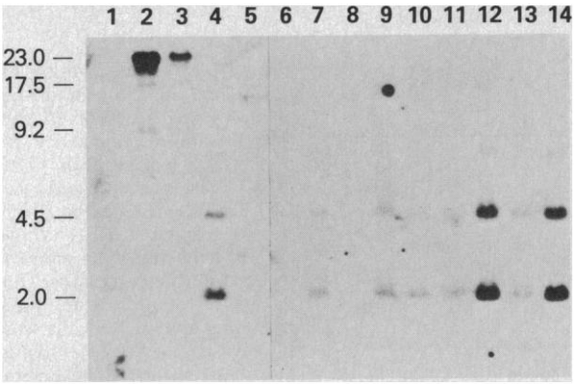



Fig. 2. Southern blotting analysis of parental and hybrid cell DNA's with the use of the pHαT2 cDNA probe specific for the α-chain of the human T-cell receptor. Lanes 1 to 3, the DNA's were digested with Eco RI. Lanes 4 to 14, the DNA's were digested with Hind III. Lane 1, LM-TK$^-$ mouse DNA (*10, 19*). Lane 2, P3HR-1 Burkitt lymphoma DNA (*10*). Lane 3, M44 Cl 2S5 hybrid DNA. Hybrid M44 Cl 2S5 contains only the 14q$^+$ chromosome of P3HR-1, Burkitt lymphoma cells with the t(8;14) translocation, and no other human chromosome (*10*). Lane 4, human MT-2 DNA. Lane 5, mouse myeloma DNA (NP3) (*10, 19*). Lanes 6 to 11, hybrid cell DNA's. Lane 6, PXB IV Cl 5 DNA. Lane 7, 77B10 Cl 28 DNA. Lane 8, DSK Cl 20-C DNA. Lane 9, 1062B44 C4 hybrid DNA (this hybrid has only a normal human chromosome 14). Lane 10, 52–63 Cl 7S17 DNA (this hybrid has only the KOP-2 14q$^+$ chromosome) (*12*). Lane 11, D69 Cl 4S7 DNA. Lane 12, P3HR-1 Burkitt lymphoma DNA. Lane 13, M44 Cl 2S5 (hybrid DNA). Lane 14, BL2 Burkitt lymphoma DNA (*19*). *Hybrid 52–63 Cl 7S17 carries the 14q$^+$ chromosome of KOP-2 cells which have a t(14;X) chromosome translocation (*12*). The breakpoint on the KOP-2 chromosome 14 is on band q32, proximally to the heavy chain locus, since hybrid 52–63 Cl 7S17 is negative for the genes for the C regions of human heavy chains (*11*).

The mouse α-chain T-cell receptor cDNA clone TT11 (*4*) was used as a probe to screen a human cDNA library derived from messenger RNA (mRNA) of the Jurkat human T lymphoma cell line (*9*). Positive clones were detected with a frequency of approximately 1 in 2000 colonies. Three clones, pHαT1, pHαT2, and pHαT3, were partially sequenced to confirm their homology to the murine α-chain T-cell clone. Extensive homology with the mouse TT11 clone was revealed in the C region and in the J region (Fig. 1). The V region of the human cDNA clones and that of the mouse α-chain clone sequenced by Saito *et al.* (*5*) showed extensive homology. Clone pHαT2 was used as a T-cell receptor α-chain specific probe to analyze a panel of DNA's derived from rodent × human somatic cell hybrids.

As shown in Table 1 and Fig. 2, segregation of hybrids bearing the α-chain DNA sequence of the human T-cell re-

Table 1. Assignment of the gene for the α-chain of the T-cell receptor to human chromosome 14.

| Hybrids | Human chromosomes | | | | | | | | | | | | | | | | | | | | | | | Tα chain gene |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | X | |
| PXBIV-Cl 5 | − | + | − | − | + | − | − | + | − | − | − | + | − | − | − | − | − | − | + | − | − | − | − | − |
| 77 B10 Cl 28 | + | − | + | − | + | − | − | − | − | − | − | − | − | + | − | − | − | + | − | − | − | − | + | + |
| DSK Cl 20-C | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | + | − | − | + | + | + | − |
| 106 2B4 4C4 | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | + |
| 52-63 Cl 7S17* | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | + |
| D69 Cl 4S7 | − | − | − | − | − | + | − | − | − | − | − | − | − | + | − | − | − | − | − | + | − | + | − | + |
| M44 Cl 2S5† | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | + |
| Nu9 | − | − | − | − | − | + | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| D2 Cl 6S5 | − | − | + | + | − | + | − | − | − | − | + | − | − | + | + | − | + | − | − | − | − | − | − | + |
| 401 AD5 EF 3-1 | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | + | − | + | + | + | − |
| 77 B10 Cl 30 | − | − | + | − | + | − | − | − | − | + | − | − | + | + | − | − | − | − | − | + | − | − | + | + |
| 77 B10 Cl 31 | + | − | + | − | + | − | − | + | + | + | − | − | + | + | − | − | − | + | − | + | − | − | + | + |
| 53-87-3 Cl 10 | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| GM54VA Cl 31 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − |
| GM × LM Cl 5 | − | − | − | + | − | − | + | + | − | − | − | − | − | − | + | − | − | − | − | + | − | + | − | − |
| D2 Cl 6S3 | − | − | + | + | − | + | − | − | − | − | + | − | − | − | + | + | + | − | − | − | − | + | − | − |
| 77 B10 Cl 5 | + | − | − | − | + | − | − | + | + | + | − | − | + | + | − | + | − | − | − | + | − | − | + | + |
| 1P1 | − | − | − | − | − | − | + | − | − | + | − | − | − | − | + | − | + | − | − | − | − | − | − | − |
| PT47 Cl 5 | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − |
| 5468 F1 Cl 1-11 | − | − | − | − | − | − | − | − | − | + | + | + | − | − | − | − | − | + | − | − | − | + | − | − |
| 5468 F2 Cl 5 | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | + | + | − |
| DSK Cl 2 | − | − | + | − | − | − | − | + | − | − | − | − | − | + | − | + | + | − | − | − | − | + | − | + |
| 706B6-40 Cl 17 | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 640-63 | − | − | − | − | − | − | − | − | + | − | − | − | + | − | − | − | − | + | − | − | + | − | − | − |
| 706-D1 | − | + | − | − | − | + | − | − | − | − | − | + | + | − | − | + | + | + | − | − | − | + | − | − |

*Hybrid 52-63 Cl 7S17 carries the 14q$^+$ chromosome of KOP-2 cells which have a t(14;X) chromosome translocation (*11, 12*). †Hybrid M44 Cl 2S5 contains only the 14q$^+$ chromosome of P3HR-1 Burkitt lymphoma with the t(8;14) chromosome translocation (*10*).

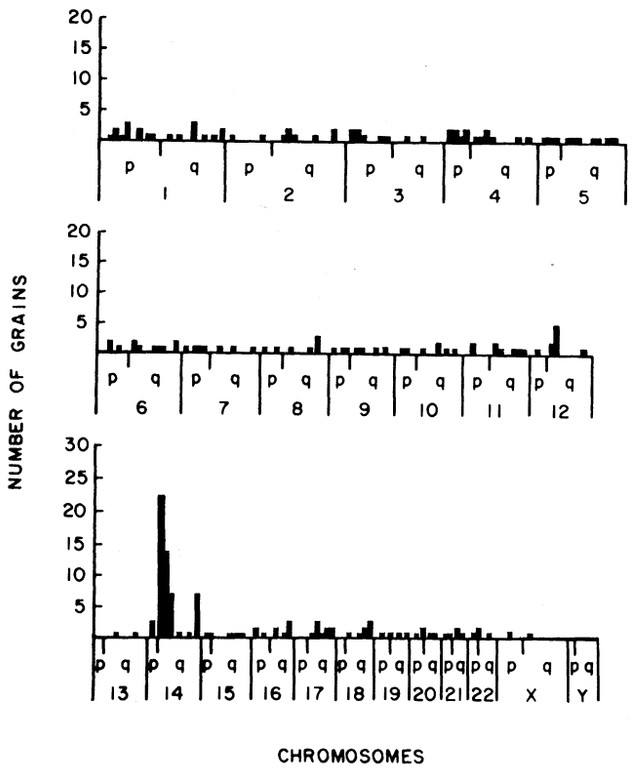



Fig. 3. Localization of the α-chain gene of the T-cell receptor in the human genome by in situ hybridization. The abscissa represents the chromosomes in their relative sizes; the ordinate shows the number of silver grains. The distribution of 227 grains on 100 cells was scored; 46 were found over 14p13–14q21 with most grains at 14q11. Chromosome 14 represents approximately 3.55 percent of the haploid genome. More than 20 percent of the grains are located on the proximal third of the long arm of chromosome 14. Thus, cytological hybridization localizes the gene for the human α-chain of the T-cell receptor to region 14q11.

ceptor was concordant with the presence of human chromosome 14. One of the hybrid clones used, M44 Cl 2S5 contained only the $14q^+$ human chromosome derived from a Burkitt lymphoma (P3HR-1) with the t(8;14) chromosome translocation (*10*). Another hybrid (52–63 Cl 7S17) contained only the $14q^+$ chromosome from human fibroblasts (KOP-2) with a t(14;X) chromosome translocation and no other human chromosome (Table 1 and Fig. 2). In both cases the breakpoints on chromosome 14 were at band q32 and both hybrids were positive for the α-chain gene of the human T-cell receptor. In one instance, with P3HR-1 cells, the breakpoint directly involved the immunoglobulin heavy chain locus separating the genes for the C and V regions of heavy chains (*10*). With (KOP-2 cells) the breakpoint involved a region immediately proximal to the heavy chain locus, since hybrid 52–63 Cl 7S17, which contained the human $14q^+$ chromosome, did not contain immunoglobulin heavy chain sequences (*11*). Since both hybrids, M44 Cl 2S5 and 52–63 Cl 7S17, contained the gene for the α-chain of the T-cell receptor, we concluded that the gene is located on chromosome 14 at a position proximal to the heavy chain locus (*12*).

Further studies of the localization of the α-chain gene on chromosome 14 with the use of in situ hybridization techniques (*13*) indicated positive hybridization of the human α-chain cDNA probe to region q11–q12 of human chromosome 14, with most grains concentrated at q11.2. We also detected a lower signal on band q32 of chromosome 14 (Fig. 3). This could be due to the partial homology between the gene for the α-chain of the T-cell receptor and the immunoglobulin heavy chain genes (*4, 5*). However, we did not observe a signal on human chromosomes 2, 22, or 7 where, respectively, the Ig κ locus, λ locus, and the gene for the β-chain of the T-cell receptor are located.

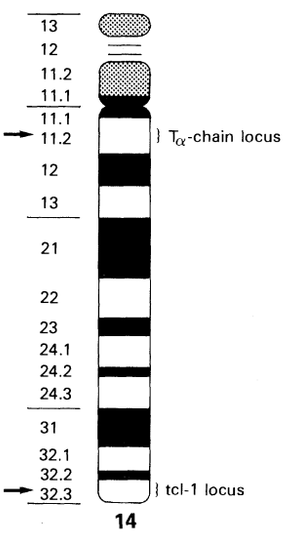



Fig. 4. Diagram of human chromosome 14. The arrows indicate the breakpoints observed in CLL cells with the inversion of chromosome 14. The brackets indicate the positions of the locus for the α-chain of the T-cell receptor (upper) and of the putative *tcl*-1 oncogene (lower). The chromosomal inversion and the t(14;14) (q11;q32) translocation should result in the juxtaposition of the T α-chain locus and of the *tcl*-1 oncogene thereby activating *tcl*-1.

It is interesting that rearrangements involving the region q11–q13 of human chromosome 14 have been observed in T-cell neoplasms (*14–16*). In acute T-cell lymphocyte leukemia (ALL), a translocation between the short arm of chromosome 11 (p13) and the long arm of chromosome 14 (q11–q13) has been identified (*14*). Similarly, reciprocal translocations between other autosomes and the same region of human chromosomes 14 have been observed in T-cell leukemias and lymphomas (*15*). An inversion of the region q11.2–q32.2 of human chromosome 14 has been observed in T-cell chronic lymphocytic leukemias (CLL) (*15, 16*). Thus it appears that the gene for the α-chain of the T-cell receptor is involved in oncogene activation following chromosomal translocations or inversions in T cells. Since T-cell ALL and T-cell lymphomas with reciprocal translocations involving the region q11.2 of chromosome 14 are characterized by translocations of segments of different human chromosomes to chromosome 14, it seems probable that the proximity to the α-chain gene results in the activation of several different proto-oncogenes. Thus it should be possible to clone the loci involved in human T-cell malignancies by exploiting their proximity to the T-cell receptor α-chain gene, as in analogous work done with B-cell malignancies carrying translocations involving the immunoglobulin heavy chain locus (*17*).

Particularly interesting are the observations that an inversion of the segment 14q11.2–q32.2 occurs in T-cell CLL and that a t(14;14) (q11;q32) translocation occurs in T-cell malignancies of patients with ataxia telangiectasia (*18*). These observations suggest that a proto-oncogene, for which we propose the name T-cell lymphoma/leukemia-1 (*tcl*-1), is located on band q32.3 of chromosome 14 and becomes activated by its proximity to the genes for the α-chain of the T-cell receptor. The mechanism for this activation would be similar to that involving the transcriptional deregulation of the c-*myc* gene in Burkitt lymphoma, where the activated c-*myc* oncogene is in close proximity to one of the three immunoglobulin loci (*19*). Thus the *tcl*-1 gene on band q32.3 of chromosome 14 may be activated by chromosome inversion or

by chromosomal translocation, either of which results in the juxtaposition of the *tcl*-1 gene and the α-chain locus (Fig. 4).

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## Atriopeptin-Immunoreactive Neurons in the Brain: Presence in Cardiovascular Regulatory Areas

Abstract. *Antisera to atriopeptin III and to a cyanogen bromide fragment of the precursor molecule atriopeptigen were prepared and used to examine the distribution of atriopeptin-like immunoreactive material in the heart and brain of the rat. Granules of this material were seen in myocytes throughout the right and left atria and were densest in the perinuclear region. The distribution of atriopeptin-like immunoreactive material in the heart is consistent with previous reports of atrial secretory granules. In the brain neurons containing the material were observed in the hypothalamus and the pontine tegmentum. Atriopeptin in the brain may serve as a neurotransmitter in neural systems controlling blood volume and composition, the same physiological functions regulated by blood-borne atriopeptin.*

It has been known for over 20 years that atrial myocytes contain secretory granules, but the function of these granules has remained obscure. Recently, peptides isolated from atria have been shown to have natriuretic and smooth muscle relaxant effects (*1*). Several of these atrial peptides (atriopeptins) have been purified and their amino acid sequences determined (*2*). The availability of synthetic atriopeptins and an isolated purified fragment of the high molecular weight precursor has allowed preparation of antisera for use in radioimmunoassays and immunohistochemistry. Using the latter method, we found that atriopeptin-like immunoreactivity is present in the atria in a pattern that implies its presence in atrial secretory granules. We also identified atriopeptin-like immunoreactive (APIr) neurons in the brain.

Two different antisera to synthetic atriopeptin III (AP III) and one antiserum to a high molecular weight cyanogen bromide fragment of the atriopeptigen precursor (HMW AP) were prepared (*3*). The specificity of these antisera was examined by measuring the displacement of [$^{125}$I]AP III by unlabeled AP III. Both antisera to AP III showed 100 percent cross-reactivity with biologically active atriopeptins I and II (*2*). However, neither showed significant cross-reactivity with two biologically inactive atriopeptin analogs: (i) the fragment of AP III consisting of amino acid residues 13 to 24 and (ii) AP III containing an inversion of the arginine-isoleucine sequence at positions 7 and 8. The antiserum to HMW AP was 100 percent cross-reactive with atriopeptins I and II. Ten percent of the binding of [$^{125}$I]AP III was displaced by equimolar concentrations of HMW AP, but the biologically inactive atriopeptin analogs were without effect.

The two antisera to AP III (ATRP1 and ATRP7) and one antiserum to HMW AP (ATRP11) were used for immunohistochemical staining of the heart (*4*). Although the staining with ATRP11 was heaviest, the pattern of APIr staining was confirmed with the two AP III antisera. There was staining of granular material in myocytes throughout the right and left atria; the granular immunoreactive material was densest in the perinuclear region (Fig. 1A). No staining was seen in the ventricle (Fig. 1, C to D). Atrial staining by ATRP11 was abolished by preadsorbing 1 ml of the diluted antiserum with 50 μg of HMW AP (Fig. 1B) and was partially blocked by preadsorption with synthetic AP III, but was not blocked by AP III-(13–24) or by AP III containing the inversion. Staining with AP III antisera was completely blocked by preadsorption with synthetic AP III but was not affected by preadsorption with either of the biologically inactive atriopeptin analogs, and was only partially inhibited by keyhole limpet hemocyanin (KLH) (*3*).

The granular distribution of APIr staining in the heart was consistent with the localization of atriopeptins in secretory granules in atrial myocytes (*1*). In electron micrographs secretory granules tend to be localized most densely in the perinuclear region of these cells (*1*), and APIr granules in our material matched this distribution. We conclude that our antisera were probably staining immunoreactive atriopeptins in atrial secretory granules.

The brain was examined for APIr staining in a series of 11 rats. In one animal, which had not been treated with colchicine, two main groups of APIr fibers were visualized with the antiserum to HMW AP. In the hypothalamus labeled fibers extended from the periventricular nucleus into the medial preoptic area, the ventral and lateral parts of the bed nucleus of the stria terminalis, the lateral hypothalamic area, and the paraventricular and arcuate nuclei. A second heavily stained bundle of fibers was seen in the pons emerging from the periventricular gray matter of the fourth ventri-