Table 2. The relationship of *R. quintana* to selected members of the α subdivision of the purple eubacteria (*11*). The ribonuclease T1-type (G ending) oligonucleotide segments contained in the *R. quintana* sequence have been compared to their counterparts determined directly from the 16*S* rRNA's of various α purple bacteria (*11*). The total number of bases in oligonucleotides (hexamer and larger) of identical composition in any pair of these catalogs is shown. The numbers in parentheses are these counts divided by 3.09; this normalization brings most of the entries in the α-2 group to values near 100 and thereby facilitates their comparison. For the α-1 (seven species) and α-3 (five species) subgroups of the α subdivision (*11*), the counts are presented as average values between each of these groups and individual species of the α-2 subgroup. Genus abbreviations not listed in Table 1 are as follows: *Rh.*, *Rhizobium*; *Rps.*, *Rhodopseudomonas*, and *Rm.*, *Rhodomicrobium*.

Species	*R. quintana*	*A. tumefaciens*	*Rh. leguminosarum*	*Rps. palustris*	*Rps. viridis*	*Rm. vannielii*
R. quintana	--------					
A. tumefaciens	362(117)	--------				
Rh. leguminosarum	377(122)	440(142)	--------			
Rps. palustris	293 (95)	294 (95)	329(106)	--------		
Rps. viridis	334(108)	321(104)	306 (99)	308(100)	--------	
Rm. vannielii	309(100)	303 (98)	295 (95)	284 (92)	337(109)	
α-1	265 (86)	247 (80)	247 (80)	226 (73)	267 (86)	252 (81)
α-3	248 (80)	263 (85)	272 (88)	238 (77)	250 (81)	230 (74)

The close relationship between the *R. quintana* and *A. tumefaciens* 16*S* rRNA sequences prompts an investigation of the possible relationships of *R. quintana* to other species in the α subdivision. Partial 16*S* rRNA sequences, in the form of oligonucleotide catalogs, exist for over 350 eubacterial species; 100 of these are from the purple bacteria (*11, 12*), and 21 from the α subdivision (*11*). This partial sequence information permits a localization of the phylogenetic position of *Rochalimaea* within the α subdivision, and it rules out previously suspected relationships to organisms such as *Xanthomonas* and *Legionella* (*12, 13*).

Table 2 shows the similarity of *R. quintana* to representatives of the three subgroups of the α subdivision, α-1, α-2, and α-3. [Similarity here is defined in terms of oligonucleotide catalogs, that is, as the number of bases in oligonucleotides (hexamer and larger) that are common to each pair of catalogs (*11*).] It can be seen that *R. quintana* is a member of the α-2 subgroup and within that unit clusters specifically with the agrobacteria and rhizobacteria.

It is interesting that the closest relatives of *R. quintana* and the rickettsia itself are all associated somehow with eukaryotic cells, often intracellularly (*14*). This correlation is perhaps made more significant by the finding that the mitochondrion too seems to have originated from within the α subdivision (*9*).

The fact that *R. quintana* groups with bacteria that are associated with plants, not with animals, suggests that rickettsiae may have arisen as plant-associated bacteria, possibly plant pathogens, and through the plant-insect bridge may have evolved to be associated with mammals. The resemblance of some plant pathogens to rickettsiae (*3*) may turn out to be more than superficial, and the finding of agrobacteria in human clinical specimens now takes on new significance (*15*).

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Coronary Vasoconstrictor Effects of Atriopeptin II

Abstract. *Atrial natriuretic peptides lower arterial pressure, cardiac filling pressure, and cardiac output. In isolated, Langendorff-perfused guinea pig hearts, atriopeptin II, the 23–amino acid atrial natriuretic peptide, is also a potent coronary vasoconstrictor. The median effective dose for atriopeptin II in guinea pig hearts is 26 nanomoles, the threshold constrictor dose is 5 nanomoles, and flow nearly ceases at a dose of 100 nanomoles in perfused hearts at constant pressure. Similar concentrations of atriopeptin II also cause coronary vasoconstriction in rat and dog heart preparations. The disulfide bridge is necessary for vasoconstrictor activity; reduction of this bridge abolishes the activity, as it does the other biological activities of atrial natriuretic peptides.*

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Mammalian cardiac atria contain secretory granules. Peptides associated with granule-rich fractions have been isolated from atria that produce natriuresis and diuresis, relax vascular and intestinal smooth muscle, and lower arterial pressure (*1*). Several of these peptides have been purified, their amino acid sequences have been determined, and they have been synthesized (*2*). Because atrial extract and atrial natriuretic peptides (ANP) relax vascular smooth muscle, it is reasonable to assume that they lower arterial pressure by relaxation of arteriolar (resistance vessel) smooth muscle (*2*). However, we have discovered that the pure 23–amino acid ANP

(*3*) atriopeptin II (APII) synthesized by one of us (H.H.S.), lowers arterial pressure by lowering cardiac output (*4*).

In conscious, standing sheep, infusion of APII into the right atrium (0.1 nmol/kg-min) lowers arterial pressure, right atrial pressure, stroke volume, and cardiac output and raises heart rate and total peripheral resistance (*4*). This hemodynamic pattern could be explained if APII directly relaxes venous smooth muscle, which in turn lowers cardiac filling pressure, stroke volume, and cardiac output. The fall in cardiac output and arterial pressure would initiate baroreflex increases in heart rate and in total peripheral resistance, but the venodilation would persist because of poor autonomic control of systemic veins. Although this explanation may account for the observed responses, it is also possible that APII directly depresses cardiac output. Thus, experiments were performed to determine if APII has direct effects on coronary or cardiac function.

The isolated Langendorff-perfused guinea pig heart preparation has been well characterized in our laboratory (*5*). Most of these experiments were performed by the method of Langendorff on eight guinea pig hearts perfused with Krebs-Hensleit solution. We determined left ventricular developed pressure (LVP), the maximum rate of change of left ventricular pressure [LV $(dP/dt)_{max}$], heart rate, coronary arterial flow, arterial pressure, arterial and venous oxygen pressure, and adenosine concentration during perfusion at constant pressure or at constant flow conditions. APII or phenylephrine were administered into the coronary flow as bolus injections. The injectates did not recirculate.

During perfusion of guinea pig hearts at constant pressure, APII caused immediate coronary vasoconstriction followed 1 to 3 seconds later by a decrease in LVP and LV $(dP/dt)_{max}$. Figure 1 depicts the dose-response curve for coronary flow in perfused guinea pig hearts at constant pressure. The threshold (5 percent) vasoactive dose of APII is 5 nmol. Probit analysis of these data revealed a significant regression of coronary flow on dose ($F = 166.78$, $P < 0.01$). The slope of the regression (in probits) is 1.17 ± 0.09. The median effective dose, ED_{50}, is 26 nmol, with 95 percent confidence limits of 10.4 to 65.3 nmol, and flow is reduced nearly to zero by a dose of 100 nmol. The decrease ($n = 4$) in coronary flow, LVP, and positive LV $(dP/dt)_{max}$ ranged from 1 ± 1 percent, 3 ± 1 percent, and 3 ± 1 percent, respectively, at 1 nmol to 93 ± 2 percent, 63 ± 6 percent, and 67 ± 1 percent, respectively, at 100 nmol. Heart rate did not change except at doses above 100 nmol, which caused intermittent bradycardia. The reduced peptide had no primary vasoactivity over the dose range studied. At 80 and 100 nmol, however, the reduced peptide did cause slight decreases in LVP, followed 10 to 14 seconds later by 20 percent decreases in coronary flow. Thus, APII's potent vasoconstrictor effect, like its other biological effects, depends on an intact disulfide bridge (*3*).

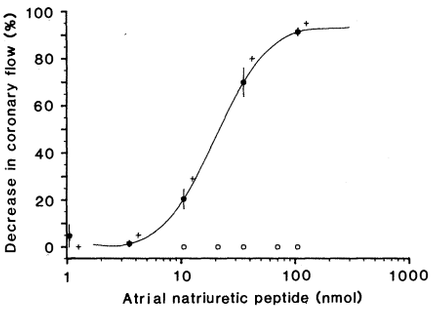

Fig. 1. Dose-response relation in guinea pig hearts for synthetic APII (Upjohn) [(●) four hearts, three or four observations per dose], synthetic APII (Peninsula) [(+) one heart], and for synthetic peptide (Upjohn) after reduction of the disulfide bond [(○) one heart]. Experiments with the Peninsula peptide and the reduced Upjohn peptide were performed to verify the purity of the synthetic Upjohn peptide.

Figure 2 shows LVP, LV $(dP/dt)_{max}$, and coronary flow in one guinea pig heart before and after administration of 100 nmol of ANP. Before the APII was administered, a bolus injection of adenosine (10 nmol) increased flow from 5.6 to 12.2 ml/min; this dose of adenosine causes almost maximal vasodilation. Norepinephrine (0.6 nmol) produced a transient, alpha-receptor–mediated vasoconstriction followed by a large increase in flow (to 11.1 ml/min) in response to its positive inotropic action. Administration of APII caused a large decrease in coronary flow followed 2 seconds later by large decreases in LVP and LV $(dP/dt)_{max}$. Administration of 10 nmol of adenosine after APII only increased flow from 2.7 to 5.0 ml/min. After the APII was administered, norepinephrine (0.6 nmol) still caused the transient decrease in coronary flow, but although LVP and LV $(dP/dt)_{max}$ increased, the positive inotropic action of norepinephrine was short-lived because coronary flow could not increase. Although adenosine and norepinephrine could not overcome APII-induced vasoconstriction, a dose of verapamil (1 μmol), which arrests the heart, caused immediate vasodilation. The magnitude of such vasodilation indicates the maximum dilation possible in this preparation after APII administration.

Perfusate gas pressures and adenosine concentrations were measured in three guinea pig hearts during constant pressure perfusion before and after two bolus injections of APII at 35 nmol and two bolus injections of APII at 100 nmol. The paired *t*-test was used to evaluate these data. In these hearts, coronary flow decreased from 4.6 ± 0.4 to 0.7 ± 0.1 ml/min-g ($P < 0.01$). The arterial *p*H, the arterial pressures of O_2 and CO_2, and the adenosine concentration were 7.41 ± 0.01, 591 ± 10 mmHg, 28 ± 1 mmHg, and 0 pmol/ml, respectively. In response to APII, the venous *p*H decreased from 7.33 ± 0.02 to 7.24 ± 0.01

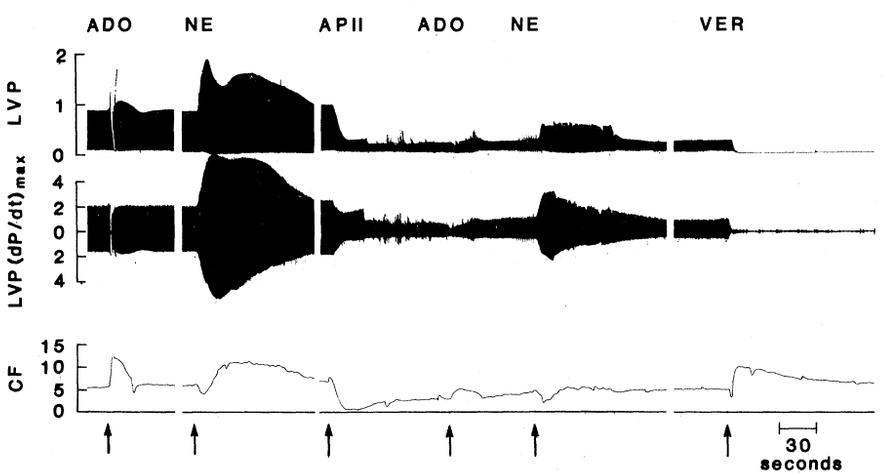

Fig. 2. Typical chart recording of LVP, LV $(dP/dt)_{max}$, and coronary flow rate (CF) during bolus injections of adenosine (ADO, 10 nmol), norepinephrine (NE, 0.6 nmol), atriopeptin II (APII, 100 nmol), and verapamil (VER, 1 μmol) in a perfused guinea pig heart at constant pressure.

Table 1. Responses of guinea pig hearts to 35 nmol of APII under constant pressure or constant flow.

Measured conditions	Constant pressure*			Constant flow†		
	Before APII	After APII	Change (%)	Before APII	After APII	Change (%)
Perfusion pressure (mmHg)	46	46		48 ± 2	71 ± 2§	49 ± 1
Coronary flow (ml/min-g)	5.5 ± 0.8	1.5 ± 0.4‡	−74 ± 4	5.0 ± 0.3	5.0 ± 0.3	
LVP (mmHg)	105 ± 5	57 ± 7‡	−53 ± 6	104 ± 4	81 ± 4§	−22 ± 4
Positive LV $(dP/dt)_{max}$ (mmHg/sec)	1618 ± 126	1058 ± 154‡	−36 ± 5	1224 ± 59	1137 ± 46	−7 ± 4
Negative LV $(dP/dt)_{max}$ (mmHg/sec)	1168 ± 49	635 ± 73‡	−46 ± 5	1050 ± 71	871 ± 75§	−17 ± 9

*n = five hearts and five replicates. †n = three hearts and seven replicates. ‡$P < 0.01$ (paired t-test). §$P < 0.05$ (paired t-test).

($P < 0.05$), the venous pressure of O_2 decreased from 163 ± 18 to 87 ± 6 mmHg ($P < 0.01$), the venous pressure of CO_2 increased from 36 ± 0.8 to 45 ± 0.4 mmHg ($P < 0.01$), and the venous adenosine concentration increased from 12 ± 2 to 27 ± 1 pmol/ml ($P < 0.01$). The changes in the pressures of perfusate gases, pH, and adenosine content are consistent with APII having a vasoconstrictor effect.

To test whether the decreases in LVP and LV $(dP/dt)_{max}$ seen in the guinea pig heart were caused by a direct negative inotropic effect of APII or were a secondary effect due to the fall in coronary flow, APII was administered to guinea pig hearts under constant flow conditions. Table 1 presents perfusion pressure, coronary flow, LVP, and the positive and negative LV $(dP/dt)_{max}$ in response to 35 nmol of APII administered under the conditions of constant pressure or constant flow. Although LVP still fell in response to APII under constant flow, the magnitude of the fall was not as great as under constant pressure. Under constant flow, the positive LV $(dP/dt)_{max}$ was not decreased significantly. The negative LV $(dP/dt)_{max}$ was significantly decreased, but this is consistent with slower relaxation during ischemia.

The differences in time courses of the vascular changes and changes in mechanical performance also support a direct vasoconstrictor effect of APII. In both perfusion at constant pressure and perfusion at constant flow, the changes in function lagged behind the vascular changes. This lag time was greater during perfusion at constant flow (4.4 ± 1.3 seconds) than during perfusion at constant pressure (1.0 ± 0.4 seconds).

Further evidence for the lack of an inotropic effect from APII is found in the phenylephrine experiments. Bolus injections of phenylephrine, a vasoconstrictor that has negligible inotropic activity, resulted in decreases in coronary flow and LVP in the guinea pig hearts that were similar to the decreases in flow and LVP produced by APII. When percent decreases in LVP (y) were plotted against percent decreases in coronary flow (x), the least-squares regressions of LVP on coronary flow were

$$y = (0.69 \pm 0.04)x - 0.8$$

for APII and

$$y = (0.61 \pm 0.11)x + 2.9$$

for phenylephrine. Phenylephrine did differ from APII quantitatively in that it was not as potent a vasoconstrictor. Over the dose range 0.1 to 30.0 nmol, phenylephrine decreased coronary flow 10 ± 2 to 31 ± 1 percent, respectively, and LVP, 5 ± 1 to 20 ± 2 percent, respectively (three hearts, 45 bolus injections). Higher doses of phenylephrine produced no greater decreases in coronary flow.

Thus, the primary action of APII on guinea pig hearts is to constrict the coronary vessels; the fall in mechanical performance is evidently secondary to the reduction and redistribution of coronary flow. Figure 3 shows that APII also is a vasoconstrictor in three rat hearts perfused with Krebs-Hensleit solution by the method of Langendorff (*5*) and in four blood-perfused dog hearts (*6*). The percent increase in coronary resistance for guinea pigs, rats, and dogs is plotted as a function of APII dose per gram of heart. A similar dose-response relation is seen for both perfusates in all three species.

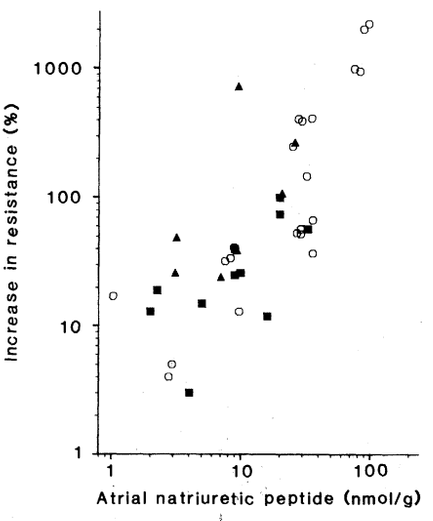

Fig. 3. Dose-response relation in three heart preparations for synthetic APII [(○) guinea pig, eight hearts; (▲) rat, three hearts; (■) dog, four hearts]. The guinea pig and rat hearts were perfused with Krebs-Hensleit solution and the dog hearts with blood.

Needleman and his co-workers (*7*) have reported selective ANP-induced renal vasodilation without coronary vasoactivity in conscious dogs and anesthetized rats. Dogs received systemic bolus injections of 5 μg/kg and rats received systemic infusions of 5 and 30 μg/kg-min. The threshold constrictor dose for the preparations reported in this study (Fig. 3) is close to 1 nmol/g or about 3 μg per gram of heart tissue. The infusions in rats (*7*) are estimated to be within one order of magnitude of the threshold dose for coronary vasoconstriction (*8*). In our experiments in conscious sheep, we found that an even lower dose of APII, 300 ng/kg-min, infused for a longer time, 30 minutes, resulted in a marked depression of cardiac output, which persisted for 30 minutes after the end of the APII infusion (*4*).

In the whole animal studies (*4, 7*), ANP are free to recirculate and relatively low rates of administration may cause large doses to be received by sensitive tissues. In contrast, in the Langendorf-perfused guinea pig and rat hearts, APII does not recirculate. It is exposed to the heart on a single pass and then eliminated from the system. It is impossible to compare the doses that are received when ANP recirculate and when they do not.

Finally, at least in cardiac atria, ANP may reach vasoconstrictor concentrations in the interstitial space when they are released. Atriopeptins may also depress cardiac function in sheep (*4*) and dogs and rats (*9*) by intramyocardial vasoconstriction and redistribution of myocardial flow at doses that do not lower total coronary flow.

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3. ANPII was synthesized by the solid-phase method of Merrifield (*10*). The detailed synthesis of APII has been described (*11*). Synthetic APII was purified by high-pressure liquid chromatography (HPLC) and Sephadex chromatography and was shown to be a single peptide identical to natural APII by thin-layer chromatography (three different solvent systems) and HPLC (four different systems, two solvents and two columns). The homogeneity of the compound was also confirmed by comparison with HPLC of reduced APII, APII after oxidation, and reduced APII obtained by treating APII with dithiothreitol. The structure of synthetic APII was confirmed by amino acid analysis (both before and after oxidation of sulfhydryl groups to disulfide), fast atom–bombardment mass spectrometry, gel filtration, and also, independently, by amino acid sequencing. In addition, the native and synthetic APII had identical effects in rabbit thoracic-aortic strips contracted with serotonin, phenylephrine, prostaglandin F_2, and Ca^{2+} and in pulmonary arteries (*12*, *13*). The cystine disulfide bridge of APII was reduced with one equivalent of dithiothreitol. The reduced APII was purified by HPLC.
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6. The dogs were anesthetized with pentobarbital sodium (30 mg/kg intravenous injection), and cannulae were placed in the femoral arteries, a femoral vein, the right atrium, and a branch of the left coronary artery. The coronary artery was perfused at constant flow rate with heparinized blood diverted from a femoral artery by a Masterflex roller pump. Coronary perfusion was adjusted until pressures were equal in the femoral and coronary arteries in the control state. The observed variable was the change in coronary perfusion pressure during bolus injection of APII into the coronary perfusion line. The weight of cardiac tissue to which APII was administered was determined by administration of crystal violet dye into the perfusion cannula and dissection and weighing of the dyed region.
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Selective Sparing of a Class of Striatal Neurons in Huntington's Disease

Abstract. *A distinct subpopulation of striatal aspiny neurons, containing the enzyme nicotinamide adenine dinucleotide phosphate diaphorase, is preserved in the caudate nucleus in Huntington's disease. Biochemical assays confirmed a significant increase in the activity of this enzyme in both the caudate nucleus and putamen in postmortem brain tissue from patients with this disease. The resistance of these neurons suggests that the gene defect in Huntington's disease may be modifiable by the local biochemical environment. This finding may provide insight into the nature of the genetically programmed cell death that is a characteristic of the disease.*

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Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder characterized by progressive dementia and chorea (*1*). Although the site of the genetic defect was recently shown to be on chromosome 4 (*2*), the pathogenesis of the disease remains unknown. The most striking neuropathological feature is severe atrophy of the neostriatum, with marked neuronal loss and gliosis (*3*). This neuronal loss is accompanied by a reduction in neurochemical markers in spiny neurons, including substance P, enkephalins, and γ-aminobutyric acid (*4*). Golgi studies show morphologic abnormalities in dendrites of spiny neurons, but the dendrites of aspiny neurons appear to be less affected (*5*).

We previously reported that concentrations of somatostatin, a neurotransmitter contained in aspiny striatal neurons (*6*), are elevated in the striatum in HD (*7*). It was unclear whether this increase was due to selective sparing of somatostatin-containing neurons or of fibers, some of which may originate from outside the striatum. The histochemical method for visualizing nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) identifies a specific subset of aspiny striatal neurons (*8*) identical to those showing immunoreactivity for somatostatin and neuropeptide Y (*9*). We confirmed this correspondence of diaphorase activity with both somatostatin and neuropeptide Y immunoreactivity in human striatum (*10*). This reaction allows a rapid and reliable visualization of neurons, with intense staining of the entire cell soma and its arborizations. We now report that cells containing NADPH-d are selectively spared in the striatum in HD.

The clinical diagnosis of HD was confirmed by neuropathologic examination. Postmortem brain tissue from four HD patients (mean age, 56 years; range, 36 to 76 years) and six age-matched controls (mean age, 59; range, 36 to 78) was dissected fresh, and 1-cm-thick coronal blocks of tissue were removed from the head of the caudate nucleus (CN) at the caudato-putaminal junction and placed in 10 percent neutral buffered Formalin at 4°C. The interval between death and dissection was less than 12 hours (HD patients, 1.7 ± 0.7 hours and controls,

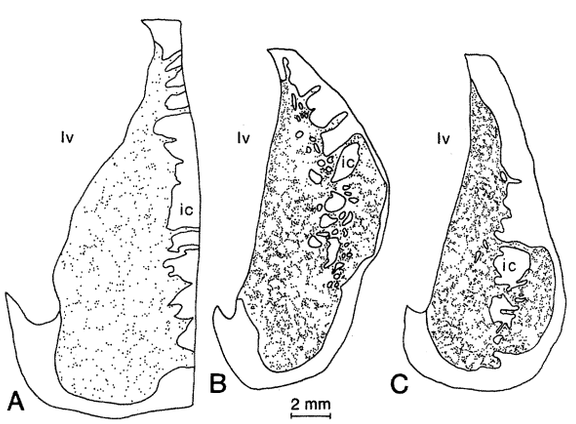

Fig. 1. Camera-lucida drawings of NADPH-d neurons (black dots), showing their distribution at the head of the CN in (A) a normal control and (B and C) two HD patients. NADPH-d neurons are distributed in a honeycomb pattern, forming bands around patches devoid of cellular staining. This pattern persists in HD, but the density of NADPH-d neurons is markedly increased. Abbreviations: ic, internal capsule and lv, lateral ventricle. Scale bar, 2 mm.