

SYMPOSIUM AND MEETING REPORTS

Cardiac and Renal Hormones

Anticancer Effects In Vitro and In Vivo

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Background: Four cardiovascular hormones, ie, vessel dilator, long-acting natriuretic peptide, kaliuretic peptide, and atrial natriuretic peptide each at 1 mmol/L, decrease up to 97% of human breast, ovarian, pancreatic, colon, kidney, and prostate adenocarcinoma cells, as well as small cell and squamous cell lung cancer cells within 24 hours.

Methods: Vessel dilator, long-acting natriuretic peptide, and kaliuretic peptide were investigated in vivo.

Results: These cardiac hormones completely stop the growth of human pancreatic adenocarcinomas in athymic mice and decrease their tumor volume by 49%, 28%, and 11%, respectively, in 1 week. When these cardiac hormones are given subcutaneously for 1 month via osmotic pumps with the pumps changed weekly, up to 80% of the human pancreatic adenocarcinomas growing in athymic mice can be completely eliminated. Similarly, two thirds of human breast cancers in athymic mice can be eliminated without surgery with these cardiac hormones. Natriuretic peptide receptors A-, B-, and C- are present on the cancer cells to mediate atrial natriuretic peptide's effects.

Conclusions: The cardiac hormones' anticancer mechanism of action(s) include a strong inhibition of mitogen (epidermal growth factor and insulin) activated extracellular signal-regulated kinases (ERK) 1/2 and as well as inhibition of basal extracellular-signal regulated kinase 1/2 and upstream MEK 1/2 phosphorylation. They cause 80% to 90% inhibition of DNA synthesis in the nucleus where these cardiac hormones have been demonstrated to localize by immunocytochemical techniques.

Key Words: cancer, natriuretic peptides, metastasis

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Cardiac hormones consist of a family of peptide hormones that are synthesized by 3 different genes and then are stored as 3 different prohormones [ie, 126 amino acid (a.a.) atrial natriuretic peptide (ANP), 108 a.a. brain natriuretic peptide (BNP), and 103 a.a. C-type natriuretic peptide (CNP) prohormones] within the heart.^{1–3} Within the 126 a.a., ANP prohormone are 4 peptide hormones (Fig. 1) whose previous known biologic properties are blood pressure regulation and maintenance of plasma volume in animals^{4–9} and humans.^{10–12}

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These peptide hormones, numbered by their a.a. sequences beginning at the N-terminal end of the ANP prohormone, consist of the first 30 a.a. of the prohormone, ie, long-acting natriuretic peptide (LANP), a.a. 31–67 (ie, vessel dilator), a.a. 79–98 (kaliuretic peptide) and a.a. 99–126 (ANP).^{13,14} The BNP and CNP genes, on the other hand, appear to each synthesize only 1 peptide hormone within their respective prohormones, ie, BNP and CNP.^{2,15–17} Each of these peptide hormones circulates in healthy humans, with vessel dilator and LANP's concentrations in plasma being 17- to 24-fold higher than ANP, 33- to 48-fold higher than BNP, and 124- to 177-fold higher than CNP.^{17–23}

Atrial natriuretic peptide inhibits smooth muscle cell proliferation (hyperplasia) as well as smooth muscle cell growth (hypertrophy).^{24–27} Atrial natriuretic peptide has growth regulatory properties in a variety of other tissues as well including brain, kidney, bone, myocytes, red blood cell precursors, and endothelial cells.^{28–35} In the kidney, ANP causes antimitogenic and antiproliferative effects in glomerular mesangial cells via inhibiting DNA synthesis.^{28,29,31}

CARDIAC HORMONES SPECIFIC DECREASE OF UP TO 97% OF CANCER CELLS WITHIN 24 HOURS

The 4 cardiac hormones from the ANP prohormone decrease the number, ie, eliminate, up to 97% of human pancreatic, colon, prostate, breast, ovarian, and kidney adenocarcinoma cells,^{36–41} angiosarcoma of the heart cells,⁴² glioblastomas of brain,⁴³ melanomas⁴⁴ as well as small-cell,⁴⁵ and squamous cell lung carcinoma cells⁴⁶ within 24 hours. There was a 97.4%, 87%, 88%, and 89% ($P < 0.001$ for each) decrease (ie, elimination) of human prostate adenocarcinoma cells secondary to vessel dilator, LANP, and kaliuretic peptide, and ANP, respectively, within 24 hours at their 1 mM concentrations, without any proliferation in 3 days after this decrease.³⁸ When used with these 4 cardiac hormones respective antibodies, their ability to decrease the number of prostate cancer cells was completely blocked indicating that their effects were specific, ie, not due to some other hormone or substance.³⁸ These cardiac hormones, as part of their mechanism of action, inhibit DNA synthesis 68% to 89% ($P < 0.001$) in these same prostate cancer cells.³⁸ Their ability to inhibit DNA synthesis is also completely blocked by their respective antibodies indicating that their effects on DNA synthesis are specific to these cardiac hormones.³⁸ Dose-response curves have revealed that there is a significantly greater ($P < 0.05$) decrease in the number of cancer cells at each 10-fold increase in the concentration of the 4 cardiac hormones (Fig. 1) synthesized by the ANP gene in human breast, colon, and prostate cancer cells as well as in small-cell and squamous cell carcinoma of lung cells.^{36–46}

BNP AND C-NATRIURETIC HAVE LESS SIGNIFICANT ANTICANCER EFFECTS

Dose-response investigations with BNP indicate that BNP has no anticancer effects at any concentration.^{38–45} C-type

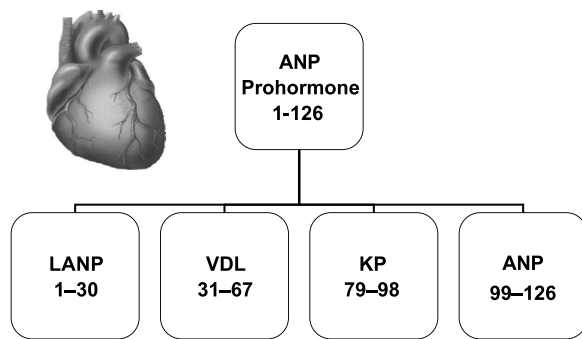


FIGURE 1. The ANP gene in the heart synthesizes a 126 a.a. prohormone with which proteolytic processing results in the formation of 4 cardiac hormones. These 4 cardiac hormones, ie, (1) LANP consists of the first 30 amino acids of the 126 a.a. prohormone, (2) vessel dilator (VDL), a.a. 31–67 of the prohormone, (3) kaliuretic peptide (KP), a.a. 79–98 of this prohormone, and (4) ANP, consisting of a.a. 99–126 of the 126 a.a. prohormone. Reprinted with permission from reference 63.

natriuretic peptide from the third cardiac gene has anticancer effects but only at 100-fold higher concentrations than that observed for the 4 cardiac hormones synthesized by the ANP gene.^{40,45} The addition of BNP for 24 hour results in 1%, 2%, and 4% (all nonsignificant) decrease in renal carcinoma cell numbers at its 1, 10, and 100 μ M concentrations.⁴⁰ With exposure to CNP for 24 hours, there was a 1% (nonsignificant), 7% (nonsignificant), and 10% ($P = 0.04$) decrease in renal carcinoma cell numbers at its 1, 10, and 100 μ M concentrations.⁴⁰

Twenty-four, 48, and 72 hours after exposure to BNP and CNP, the amount of decrease in renal carcinoma cells with these 2 peptides was similar to that which occurred at 24 hours.⁴⁰ Thus, at 48 hours (ie, 24 hours after no exposure to these peptides), there was 1%, 3%, and 5% decrease (all nonsignificant) with 1, 10, and 100 μ M of BNP, and a 2%, 6%, and 9% decrease in renal cancer cell numbers with CNP. At 72 hours, there was 1%, 2%, and 5% decrease in renal cell number (all nonsignificant) secondary to 1, 10, and 100 μ M of BNP, whereas the renal carcinoma cancer cell numbers decreased 1%, 7%, and 9% with 1, 10, and 100 μ M, respectively, of CNP.⁴⁰ The cell numbers were identical at 96 hours for BNP with 1%, 2%, and 5% decrease and for CNP with a 1%, 7%, and 9% decrease at their 1, 10, and 100 μ M concentrations.⁴⁰

URODILATIN, A KIDNEY HORMONE, IN ADDITION, HAS ANTICANCER EFFECTS

Urodilatin is a peptide hormone formed by a differential processing of the ANP prohormone in the kidney, as opposed to all other tissues, where instead of cleaving the 126 a.a. prohormone between a.a. 98 and 99 to form ANP and kaliuretic peptide, it cleaves this prohormone between a.a. 95 and 96.^{14,47–49} The cleavage of the ANP prohormone in the kidney results in 4 a.a. from the C-terminal end of kaliuretic peptide (ie, threonine-alanine-proline-arginine) being attached to the N-terminus of ANP with the resultant peptide called urodilatin.^{3,14,47–49} It is important to note that the amino acids in urodilatin are identical to the 4 C-terminal a.a. of kaliuretic peptide and identical to all the a.a. in the ANP portion of this kidney peptide.^{3,14,47–49} Atrial natriuretic peptide, BNP, CNP, and urodilatin are ring-structured peptides linked by cysteine bonds that bind to the same natriuretic peptide receptors (NPR) -A, -B, and -C but with a different affinity for each of these receptors.⁵⁰ Atrial natriuretic peptide

binds to both the NPR-A and C-receptors with a higher affinity than BNP or CNP.⁵⁰ The binding to the NPR-A receptor is ANP>BNP>>CNP, whereas binding to the NPR-C receptor is ANP>CNP>BNP (50). C-type natriuretic peptide binds to NPR-B receptor with a higher affinity than BNP or ANP.⁵⁰ Urodilatin's binding to these receptors is of a similar affinity to ANP.^{51–53} Thus, one might expect that urodilatin may have anticancer effects as this peptide has identical a.a. to ANP and identical a.a. to the 4-terminal a.a. of kaliuretic peptide, both of which have anticancer effects. Urodilatin decreased the number of renal carcinoma cells 66% at its 100- μ M concentration, whereas ANP and kaliuretic peptide with the same amino acids at this same concentration eliminated 70% and 74% of the renal carcinoma cells in 24 hours.⁴⁰ Urodilatin, vessel dilator, LANP, kaliuretic peptide, and ANP each at their 1- μ M concentrations inhibit DNA synthesis when incubated with the human renal carcinoma cells for 24 hours by 65%, 84%, 70%, 74%, and 77%, respectively ($P < 0.001$).⁴⁰ Thus, urodilatin has significant anticancer effects in vitro on human renal cell carcinoma cells and inhibits DNA synthesis in this cancer cell similar to ANP and kaliuretic peptide.

CARDIAC NATRIURETIC HORMONES STOP THE GROWTH OF HUMAN PANCREATIC ADENOCARCINOMAS IN VIVO

The first cancer studied both in vitro and in vivo was human pancreatic adenocarcinomas which have the lowest 5-year survival rate of all common cancers.^{54,55} The 5-year survival rate of persons with adenocarcinoma of the pancreas is 1% with a median survival of only 4 months.^{54,55} Current cancer chemotherapy and surgery prolong survival by a few months but the previously mentioned 4-month mean survival is for persons treated with surgery and/or current cancer chemotherapeutic agents.^{54,55}

In vivo, the peptide hormones from the ANP prohormone have impressive effects as anticancer agents. Vessel dilator (139 ng/min/kg of body weight) infused subcutaneously for 14 days via osmotic pumps completely stopped the growth of human pancreatic adenocarcinomas in athymic mice ($n = 14$) with a decrease in their tumor volume, even when the tumor volume was large, ie, 60-fold increase in size over basal palpable tumor before peptide infusion was begun,⁵⁶ to mimic what occurs in humans, ie, the pancreatic adenocarcinomas in humans are usually large before they are discovered.^{57,58} The tumor volume increased 69-fold in this 2-week period ($P < 0.001$) when measured with electronic Vernier calipers in the placebo ($n = 30$)-treated mice.⁵⁶ Dose-response studies revealed that at concentrations as low as 1.7 ng/min/20 g mouse, vessel dilator could completely stop the growth of the human pancreatic adenocarcinomas, but at this concentration, there was no decrease in the volume of the tumor by vessel dilator.⁵⁶ The tumor volume of the untreated human pancreatic adenocarcinoma increased 172-fold in 3 weeks and increased 300-fold 4 weeks after the tumors first became palpable.⁵⁶ After 2 months, the volume of the untreated human pancreatic adenocarcinoma was 1306-fold greater than when the tumors first became palpable.⁵⁶ When these peptide hormones at 10-fold higher concentrations (ie, at 1.4 μ g/min/kg body weight) were infused for 4 weeks, in addition to completely stopping the growth of this aggressive adenocarcinoma, vessel dilator, LANP, and kaliuretic peptide decreased human pancreatic adenocarcinomas' tumor volume after 1 week by 49%, 28%, and 11%, respectively, with a 1- and 20-fold increase in the tumor volume in ANP- and placebo-treated mice.⁵⁶ Cyclic 3', 5' guanosine monophosphate (GMP) (0.05 μ g/min/20 g mouse body weight)

inhibited after 1 week the growth of this cancer 95%.⁵⁶ There was no evidence of cytotoxicity in any of the normal tissues during the infusion of these peptide hormones.

The above in vivo studies were done with subcutaneous osmotic pumps in place (ie, were not changed) for 4 weeks. Because of the possibility that the peptide hormones may have been degraded at body temperature for 4 weeks, the above in vivo experiments were repeated with the osmotic infusion pumps changed weekly.⁵⁷ When each of these peptides at 3 nM min⁻¹ kg⁻¹ body weight were infused subcutaneously for 28 days in athymic mice bearing human pancreatic adenocarcinomas, ANP eliminated 80% the human pancreatic cancers.⁵⁷ Vessel dilator, LANP, and kaliuretic peptide eliminated the primary pancreatic cancers in 33%, 20%, and 14% of their respective treatment groups.⁵⁷ In none of the animals in which the pancreatic adenocarcinomas were eliminated in the primary site did, a single animal ever had a recurrence in the primary site.⁵⁷ One ANP-treated animal developed a metastatic lesion, and this lesion was eliminated with treatment with vessel dilator.⁵⁷ Even the treated animals which did not have a total cure, their tumor volume decreased to less than 10% (and with vessel dilator to less than 2%) of that of the untreated animals both during treatment and in a 6-month follow-up period.⁵⁷

CARDIAC HORMONES ELIMINATE TWO THIRDS OF HUMAN BREAST CANCERS IN ATHYMIC MICE

It is estimated that in 2008, there will be an estimated 184,450 new cases of breast cancer and 40,930 deaths from breast cancer in 2008 in the United States.⁵⁸ Breast cancer is the second leading cause of death from cancer in women and the leading cause of death in women aged 40 to 55 in the United States.⁵⁹ Breast cancer is the leading cause of cancer death in women worldwide.⁵⁹ The number of new cases of breast cancer worldwide was estimated to be 1.05 million with 370,000 deaths in 2000.⁵⁹ Obviously, there is a need for new therapies for breast cancer.

Vessel dilator and kaliuretic peptide each eliminated 67% (2 out of every 3 treated) human breast cancers in athymic mice without any surgery.⁶⁰ Long-acting natriuretic peptide and ANP eliminated 50% and 33%, respectively, of the human breast cancers in their respective groups (Fig. 2). There was no recurrence of the breast cancers in the primary site and no metastasis except in the ANP-treated group in 1 year posttreatment.⁶⁰ The NPR-A and -C were decreased 50% and 31%, respectively, in metastatic versus primary ANP-treated breast adenocarcinomas.⁶⁰

CARDIAC HORMONES MECHANISM OF ACTION: INHIBITION OF THE ACTIVATION OF EXTRACELLULAR SIGNAL-REGULATED KINASE 1/2

Extracellular signal-regulated kinase (ERK) 1/2 is a mitogen-activated protein kinase (MAPK) important for the growth of cancer(s).^{61,62} Growth factors such as epidermal growth factor (EGF), fibroblast growth factor, platelet-derived growth factor, and vascular endothelial growth factor after binding to their specific receptor tyrosine kinases work via ERK 1/2 kinase to cause proliferation.⁶¹ Epidermal growth factor, for example, when it binds to its EGF receptor, causes this receptor to autophosphorylate on tyrosine residues and recruits the Grb2-Sos complex to turn on membrane-associated Ras, which then activates the Ras/Raf-Mek 1/2-ERK 1/2 kinase cascade.⁶¹ Of the MAPKs, ERK 1 and 2, 42, and 44 kDa proteins, can directly translocate to the nucleus and stimulate DNA synthesis and the production of several intermediate early

genes such as *c-fos* and *c-myc*, which are implicated, causing cells to divide and grow.^{61,62}

Vessel dilator and kaliuretic peptide decrease the activation of ERK 1/2 over a concentration range of 0.01 μ M to 1 μ M. Vessel dilator and kaliuretic peptide (each 1 μ M) inhibit the phosphorylation of ERK 1/2 kinase 96% ($P < 0.0001$) and 70% ($P < 0.001$), respectively (Fig. 3).⁶³ Both have significant effects within 5 minutes at their 0.01- μ M concentrations. The inhibition of ERK 1/2 lasted for at least 2 hours secondary to both.⁶³ Their ability to inhibit ERK 1/2 was inhibited by cyclic GMP antibody, and cyclic GMP itself inhibited ERK 1/2 phosphorylation.⁶³ Vessel dilator and kaliuretic peptide both inhibit ERK 1/2 kinase mediated via cyclic GMP as part of their anticancer mechanism(s) of action.

Atrial natriuretic peptide and LANP, likewise, decrease the activation of ERK 1/2 over a concentration range of 0.01 μ M to 10 μ M.⁶⁴ Atrial natriuretic peptide and LANP's maximal inhibition the phosphorylation of ERK 1/2 kinase were 94% and 88% ($P < 0.0001$), respectively.⁶⁴ Atrial natriuretic peptide had significant effects within 5 minutes at its 10- μ M concentration. The inhibition of ERK 1/2 lasted for at least 2 hours, where it was maximal, secondary to ANP and LANP. Their ability to inhibit ERK 1/2 was inhibited by cyclic GMP antibody and cyclic GMP itself inhibited ERK 1/2 phosphorylation suggesting that cyclic GMP mediates their effects of inhibiting the phosphorylation of ERK 1/2.⁶⁴

Growth-promoting hormones such as insulin and EGF work as mitogens via ERK 1/2 MAPK to cause growth.^{61,62} Insulin (1 μ M) and EGF (10 ng/ml) each enhance the phosphorylation of ERK 1/2 by 66%.⁶⁵ This enhanced phosphorylation of ERK 1/2 was decreased down to 10%, 8%, 27%, and 13% above nonstimulated ERK 1/2 by vessel dilator, kaliuretic peptide, LANP, and ANP.⁶⁵ Epidermal growth factor's 66% enhanced phosphorylation of ERK 1/2 was inhibited to a minus 11% (ie, 117% decrease) plus 4%, 13%, and 16% by vessel dilator, ANP, LANP, and kaliuretic peptide, respectively, compared with nonstimulated ERK 1/2 activity.⁶⁵ Using the respective antibodies to these 4 cardiac hormones completely blocked their ability to inhibit the phosphorylation

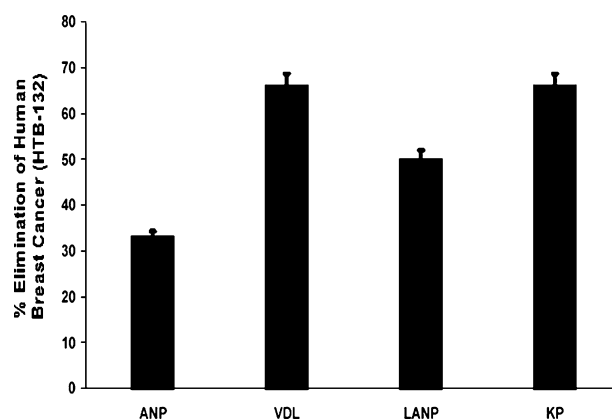


FIGURE 2. Elimination of human breast adenocarcinomas with cardiac hormones. The graph for each peptide hormones indicates the percent of complete elimination of the HTB-132 human breast adenocarcinomas in female athymic mice. The elimination of human breast cancer with vessel dilator, kaliuretic peptide, and LANP were significant at $P < 0.0001$, whereas ANP was significant at $P < 0.01$ when evaluated by analysis of variance with repeated measures design for within group comparisons. Reprinted with permission from 60.

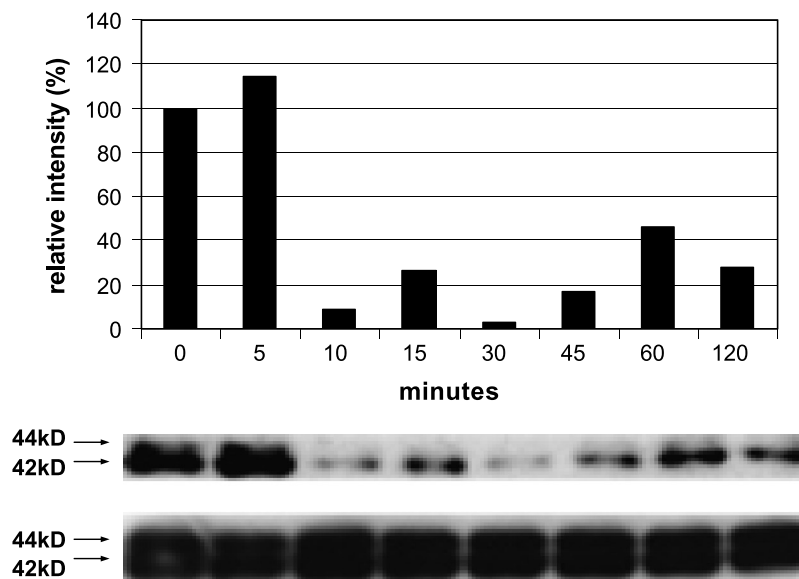


FIGURE 3. Vessel dilator at 1 μ M inhibits 96% of the phosphorylation of ERK 1/2 at 30 minutes which was significant at $P < 0.0001$ when evaluated by analysis of variance. At 2 hours, there was still a significant ($P < 0.001$) 69% decrease in the activation of ERK 1/2 kinase when evaluated by analysis of variance. ERK 1 is at 44 kDa, whereas ERK 2 is at 42 kDa. (B) Phosphorylation of ERK 1 and 2 at Try-204. (C) Total ERK 1 and 2. The relative intensity in the bar graphs is a comparison against untreated ERK 1/2 (100% intensity). Reprinted with permission from reference 63.

of ERK 1/2 by insulin and EGF, indicating that their effects are specific.⁶⁵

CARDIAC HORMONES INHIBIT THE ACTIVATION (PHOSPHORYLATION) MITOGEN-ACTIVATED PROTEIN KINASES MEK 1/2

The Ras/Raf/MEK/ERK (MAPK) signaling pathway is very important for the development of cancers and is constitutively activated in many types of cancer.⁶⁶ This pathway is frequently constitutively activated in prostate and breast cancer, and its increased expression is associated with a poor prognosis.⁶⁷ A family of protein kinases located upstream of the MAPKs (ERK 1/2) and responsible for their activation are the MAPK kinases.⁶⁸ The prototype member of this family, designated MAPK kinase (MKK-1)/or MEK-1, specifically phosphorylates the MAPK regulatory threonine and tyrosine residues present in the Thr-Glu-Tyr motif of ERK 1/2.^{68,69} A second MEK family member, namely MEK-2, resembles MEK-1 in its substrate specificity but is 7 residues longer than MEK-1 with its amino acid sequence being 81% identical to MEK-1.⁶⁹

Vessel dilator and kaliuretic peptide decreased the activation of MEK 1/2 over a concentration range of 0.01 μ M to 10 μ M in human prostate cancer cells.⁷⁰ Vessel dilator and kaliuretic peptide (each 10 μ M) inhibited the phosphorylation of MEK 1/2 kinase by 98% ($P < 0.0001$) and 81% ($P < 0.001$), respectively.⁷⁰ Atrial natriuretic peptide and LANP decreased the activation of MEK 1/2 over a concentration range of 0.01 μ M to 10 μ M in human prostate cancer cells.⁷¹ Long-acting natriuretic peptide and ANP (each 10 μ M) inhibited the phosphorylation of MEK 1/2 kinase 97% ($P < 0.00001$) and 88% ($P < 0.00001$), respectively.⁷¹ The inhibition of MEK 1/2 was maximal at 2 hours, and ceased by 4 hours, secondary to all 4 peptides.^{70,71} The ability of peptides to inhibit MEK 1/2 was inhibited by cyclic GMP antibody and cyclic GMP itself inhibited MEK 1/2 phosphorylation by 93%.^{70,71} This would suggest that the cardiac hormones ability to decrease the phosphorylation of MEK 1/2 is mediated by the intracellular mediator cyclic GMP (Fig. 4).^{70,71}

VESSEL DILATOR AND KALIURETIC PEPTIDE INHIBIT RAS

In a series of preliminary investigation, vessel dilator inhibits 95% of the activation (ie, phosphorylation) of Ras-GTP (ie, the active form of Ras kinase), whereas kaliuretic peptide inhibits 90% of Ras (unpublished observations). Their effects on Ras are mediated at least in part by the intracellular messenger cyclic GMP (unpublished observations). Thus, these peptide hormones have 3 metabolic targets in cancer cells, r.e. Ras, MEK 1/2, and ERK 1/2 of the Ras-MEK 1/2-ERK 1/2 kinase cascade (Fig. 5).

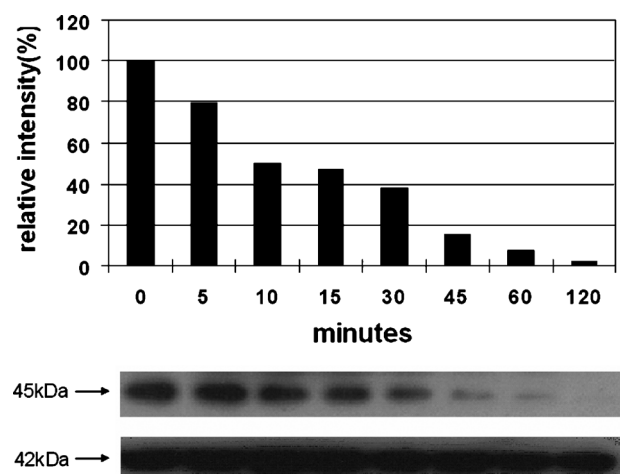


FIGURE 4. Vessel dilator at 10 μ M inhibits 98% of the phosphorylation of MAPK kinase (MEK 1/2), which was maximal at 2 hours and significant at $P < 0.00001$ when evaluated by analysis of variance. MEK 1/2 is at 45 kDa, whereas B-actin (loading control) is at 42 kDa. The relative intensity in the bar graphs is a comparison against untreated MEK 1/2 (100% intensity). Reprinted with permission from Sun et al.⁷⁰

CARDIAC HORMONES' MECHANISM OF ACTION: RECEPTORS

Each of the human cancer cells listed above have NPRs to mediate these peptide hormones' effects.^{36–46} Thus, when human breast and kidney adenocarcinoma cells were evaluated by Western blots, NPR-A, -B and -C were demonstrated to be present.^{37,40} The breast adenocarcinoma cells have developed an NPR-A- and -C receptors to mediate ANP's effects via membrane-bound guanylate cyclase which is part of the NPR-A receptor and separately via NPR-C receptor-mediated mechanisms.⁷² The NPR-C receptor does not contain guanylate cyclase which catalyzes the formation of the intracellular messenger cyclic GMP. Atrial natriuretic peptide's signaling via the NPR-C receptor is thought to involve a cascade of Ca^{2+} influx, activation of endothelial nitric oxide synthase with resulting formation of nitric oxide activating cytosolic guanylate cyclase, which in turn, increases the concentration of cyclic GMP.⁷² The presence of these receptors helps to explain why ANP, but not BNP and CNP, has effects at its 1- μM concentration as ANP binds to both receptors with a stronger affinity than BNP or CNP and, thus, a lower concentration of ANP is needed to have effects.⁵⁰ When the concentrations of CNP and BNP are increased 100-fold, in dose-response curves, CNP, but not BNP, has effects of decreasing the number of cancer cells.^{40,45} This is consistent with CNP's binding to NPR-C receptor with a stronger affinity than BNP but not as strong as ANP, ie, binding to NPR-C receptors is $\text{ANP} > \text{CNP} > \text{BNP}$.⁵⁰

CARDIAC HORMONES' MECHANISM OF ACTION: INHIBITION OF DNA SYNTHESIS

Vessel dilator, LANP, kaliuretic peptide, and ANP each at their 1- μM concentrations inhibit DNA synthesis when incubated with pancreatic adenocarcinoma cells for 24 hours by 91%, 84%, 86%, and 83%, respectively ($P < 0.001$ for each).³⁶ One of the known mediators^{73,74} of these peptide hormones' mechanism(s) of action, ie, cyclic GMP, inhibited DNA synthesis in these adenocarcinoma cells by 51%.³⁶ Dose-

response curves revealed that 8-bromo- cyclic GMP, the cell-permeable analog of 8-bromoguanosine 3', 5'-cyclic monophosphate, decreased DNA synthesis in these cancer cells 46%, 42%, 39%, and 34% (all $P < 0.05$) at its 3 mM, 1 mM, 100 μM , and 1 μM concentrations, respectively.³⁶ Even at 1 nM (ie, 10^{-9} M) of 8-bromo-cyclic GMP, there was a 25% decrease in DNA synthesis in the adenocarcinoma cells ($P < 0.05$).⁴⁶ At 100 pM of 8-bromo cyclic GMP, its effects on DNA synthesis in these adenocarcinoma cells became nonsignificant (14% decrease).

These 4 cardiac hormones inhibit DNA synthesis 80% to 90% in all human cancer cell lines.^{36–46} Thus, after inhibiting ERK 1/2, DNA synthesis (a further or final step in the Ras-ERK 1/2 pathway) is inhibited within the nucleus. These peptide hormones ability to inhibit DNA synthesis is specifically mediated by the intracellular mediator cyclic GMP, whereas when a cyclic GMP antibody is incubated with the cardiac hormones, they are unable to inhibit DNA synthesis (Fig. 5).³⁹

LOCALIZATION OF CARDIAC HORMONES TO THE NUCLEUS OF PANCREATIC ADENOCARCINOMAS

All 4 of these cardiac hormones synthesized by the prohormone *ANP* gene localize to the nucleus of human pancreatic adenocarcinomas by immunocytochemical techniques, where they can inhibit DNA synthesis.^{75,76} These are the first antigrowth peptide hormones that have been demonstrated to localize to the nucleus of cancers, and all 4 cardiac hormones localize to the nucleus.^{75,76} These 4 cardiac hormones also localized to the capillaries growing into the human cancers and the cytoplasm and fibroblasts within the tumors.^{75,76}

CONCLUSIONS

- Atrial natriuretic peptide gene synthesizes a 126 a.a. prohormone which contains 4 peptide hormones, ie, LANP, vessel dilator, kaliuretic peptide, and ANP.
- These 4 cardiac hormones eliminate up to 97% of human pancreatic, colon, breast, ovarian, renal, and prostate adenocarcinomas as well as glioblastomas of brain, angiosarcoma of breast, melanomas, small-cell, and squamous cell lung carcinomas in vitro.
- These 4 cardiac hormones eliminate up to 80% of human pancreatic adenocarcinomas and 2/3 of human breast cancers in vivo in athymic mice.
- The mechanism of action in cancer cells involves inhibition of up to 95% of Ras, 98% of MEK1/2 and 97% of ERK 1/2 in the Ras-MEK 1/2-ERK 1/2 kinase cascade (Fig. 5) and up to 91% of DNA synthesis mediated via the intracellular messenger cyclic GMP.

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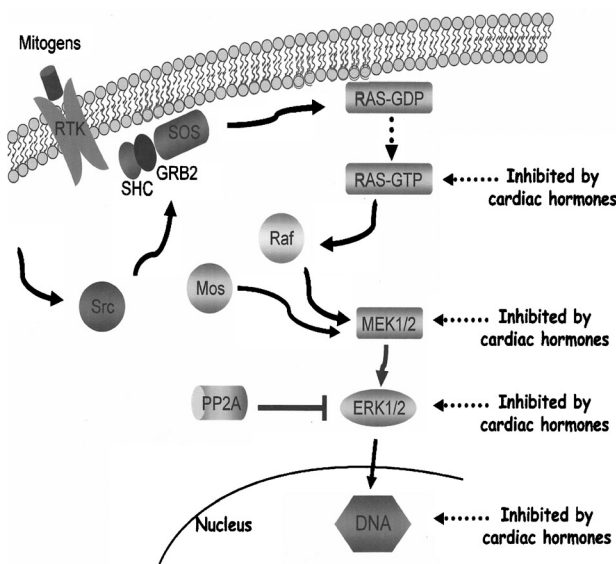


FIGURE 5. Cardiac hormones inhibit 3 metabolic targets, ie, Ras-GTP, MEK 1/2, and ERK 1/2 of the Ras-MEK 1/2-ERK 1/2 kinase cascade by 95% to 98%. They are also strong inhibitors (ie, 91%) of DNA synthesis within cancer cells.

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