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New paradigms in heart-brain medicine: Nonlinear physiology, state-dependent proteomics

Two pivotal studies using a conscious pig model of heart attack suggest new paradigms for the discovery of cardiac devices and drugs. The first study[1] showed that nonlinear analysis of heartbeat intervals, which are controlled by the nervous system, could predict with high sensitivity and specificity whether or not ventricular fibrillation (VF) would later occur following occlusion (90% reduction of blood flow by pulsed-Doppler recording) of the left anterior descending coronary artery. This predictive ability was later confirmed by retrospective data from humans exhibiting coronary artery narrowing and nonsustained ventricular tachycardia.[2] The second pivotal study[3] showed that the state-dependent release of a salutary molecule during rapid eye movement (REM) sleep would suppress arrhythmogenesis in the acutely infarcted heart. It is presumed that neurosecretion explains the salutary effect, as the latency is on the order of seconds.

This article reports previously patented data from additional investigations by me and my colleagues to further explore these two new paradigms in heart-brain medicine. First I review results from a prospective multicenter study of heartbeat analysis using a nonlinear algorithm to predict future arrhythmic death in emergency room patients. Then I discuss insights from state-dependent proteomics in the hibernating woodchuck and resulting efforts to isolate, identify, and synthesize an anti-infarction molecule from the woodchuck and test its efficacy using bioassay models.

NONLINEAR ANALYSIS OF HEARTBEAT INTERVALS

Background and methods

Heartbeat intervals were assessed using the PD2i nonlinear algorithm, details of which are provided in three published patents.[4–6] In brief, the algorithm produces a descriptor of deterministic chaos in the heartbeat, the "point correlation dimension" (PD2i) of interbeat intervals.[1,2] This nonlinear algorithm, unlike others in the field, does not require random data variation or stationary data.

Using a prospective, multicenter design, we tested the nonlinear PD2i algorithm in 400 patients presenting in the emergency room with chest pain. All patients were determined to be at high cardiac risk (> 7% probability of acute myocardial infarction [MI]) by the protocol developed by Lee et al[7] to rule out low-risk patients. All patients had 15-minute electrocardiograms (ECGs) recorded, each of which was digitized at 1,000 Hz. Hinkle-Thaler criteria[8] for suddenness and unexpectedness of out-of-hospital death were used to adjudicate arrhythmic death (AD) during 1-year follow-up. This is the first preliminary report of our study results, which have been submitted for archival publication.[9]

Results

All-cause mortality at 1-year follow-up was 10.2%, with 7.6% of cases judged to be AD. Many of the cases of AD occurred in subjects without a history of MI or a hospital diagnosis of acute MI.

Nonlinear PD2i analysis of the heartbeat intervals recorded in the emergency room accurately predicted patients who later died of arrhythmic death (AD, documented VF, or fulfillment of the Hinkle-Thaler criteria):

- For predicting AD at 30 days, the algorithm had a sensitivity of 100%, a specificity of 56%, and a relative-risk statistic >13.5 ($P \leq .001$)
- For predicting AD at 360 days, the algorithm had a sensitivity of 95%, a specificity of 57%, and a relative-risk statistic >22.8 ($P \leq .001$).

Figure 1 shows the R-R intervals and corresponding PD2i values for 18 AD patients who died suddenly and 18 matched controls who had an acute MI but who survived through at least the 1-year follow-up period. It is readily apparent that all of the AD subjects had time-dependent PD2i excursions and that

* Dr. Skinner reported that his employer, Vicor Technologies, holds patents for the nonlinear PD2i algorithm discussed in this article and has applied for patents on molecules discovered with the state-dependent proteomics method.

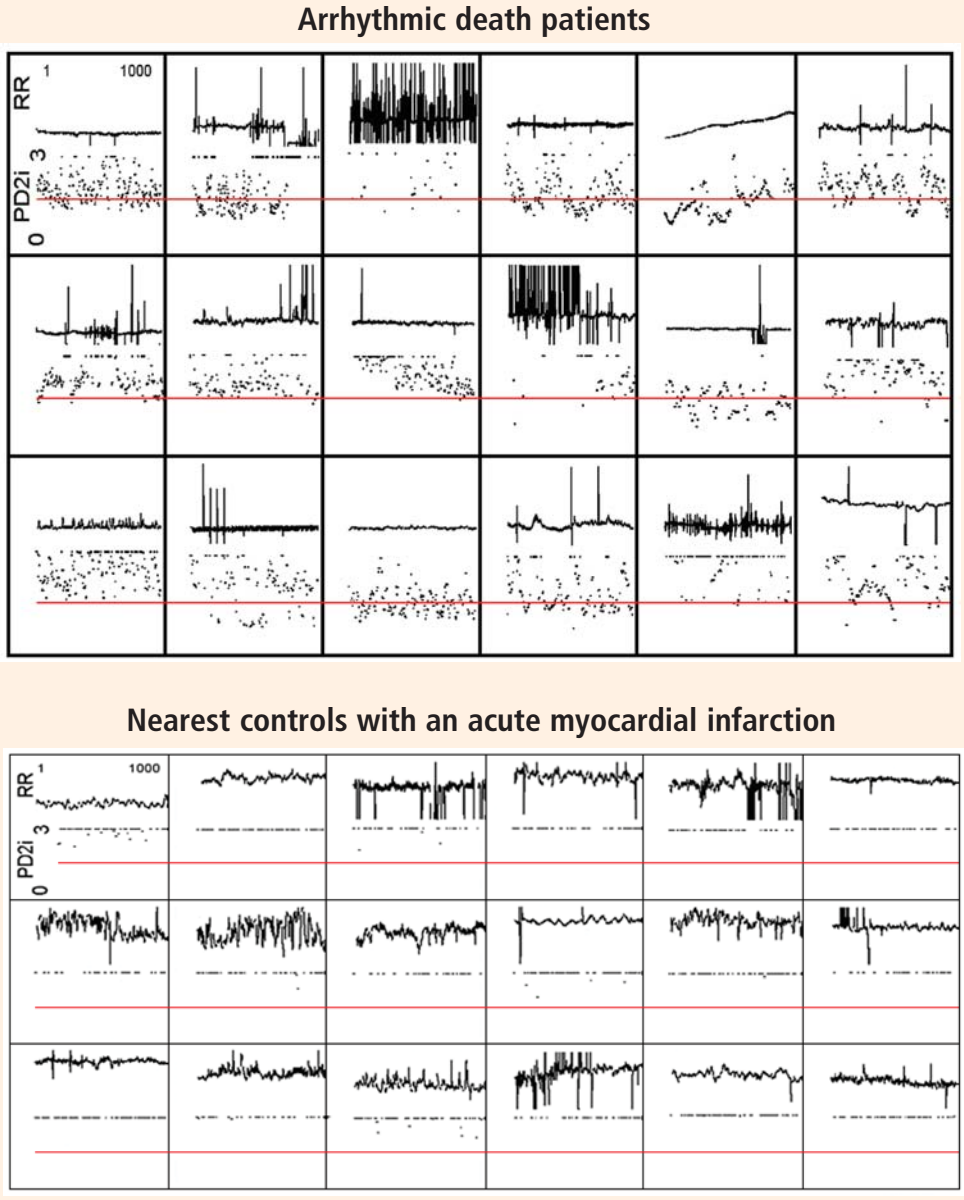

FIGURE 1. R-R intervals and corresponding PD2i values for 18 arrhythmic death (AD) patients and 18 control patients with acute myocardial infarction (MI) in a prospective emergency room study of patients with chest pain.[9] The AD patients all exhibited low-dimensional excursions of PD2i values, with most values less than 3.0. The acute MI controls did not manifest AD over 1 year of follow-up and did not exhibit low PD2i excursions. Red lines indicate a PD2i of 1.4, which served as the best separator between AD patients and controls.

the controls did not. Most of the PD2i values in the AD patients were less than 3.0, whereas most of these values in the controls with acute MI were greater than this level (shown on the line at 3 in **Figure 1**). The best separator between the two groups, as in our previous retrospective study,[2] turned out to be a PD2i value less than or equal to 1.4 (red lines in **Figure 1**).

The relative-risk statistic is thought to be the best measure for prospective clinical studies, as it emphasizes the ratio of true-positive predictions (which physicians want to make) to false-negative predictions (which physicians never want to make). **Table 1** presents the relative-risk statistics for the PD2i measure of heartbeat intervals compared with those of other algorithms previously proposed as predictors of AD. As shown in the table, the PD2i has a highly statistically significant superiority in predictive ability relative to the other algorithms across all patient subgroups. The comparative measures include other nonlinear algorithms (DFA, ApEn, 1/f Slope), all of which require stationary data, and the more common linear ones based on the stochastic model (LF/HF power, LF(ln)

power, MNN, and SDNN), all of which require stationary data *and* random variation (see **Table 1** for expansions of algorithm abbreviations).

Figure 2 depicts the PD2i values of heartbeat intervals in relation to refractoriness, as expressed by T waves, in three patients who manifested AD at various times following ECG recordings. In **Figure 2A**, the patient went into documented VF within minutes after the ECG. At the right are shown three samples of five successive heartbeats in which the large R waves and their preceding PQ intervals are aligned. There is an apparent lability (L) in the T waves throughout the period of recording. In **Figure 2B**, the patient did not manifest AD until 2 days after the ECG. Lability of the T waves did not begin until the PD2i transiently descended to the vicinity of 1.4 (horizontal line). In **Figure 2C**, the patient did not manifest AD until 2 weeks after the ECG. Note that there is no T-wave lability in this patient yet there are still PD2i excursions below 1.4 that predict the subsequent AD. These results show that the PD2i values are not related to T-wave lability per se, but it can appear this way in some patients (eg, **Figure 2B**).

TABLE 1
Relative-risk statistics for arrhythmic death at 1 year for various linear and nonlinear algorithms applied to the same prospective data set

	Patient subgroups			
Algorithm	AMI	Non-AMI	Post-MI	Non-post-MI
PD2i	> 7.39*	> 12.17*	> 4.51*	> 16.85*
DFA	0.70	0.44	0.63	0.48
1/f Slope	1.67	0.56	0.87	0.90
ApEn	0.50	1.44	0.00	0.72
SDNN	0.68	1.75	0.83	1.34
MNN	1.94	> 20.82*	3.00	> 3.61†
LF/HF	1.08	0.66	2.52	0.61
LF(ln)	1.08	> 5.13†	0.73	2.09

* $P < .01$, Fisher's exact test

† $P < .05$, Fisher's exact test

AMI = acute myocardial infarction; MI = myocardial infarction; PD2i = point correlation dimension; DFA = detrended fluctuation analysis; 1/f Slope = 1/f slope of power spectrum; ApEn = approximate entropy; SDNN = standard deviation of normal to normal beat intervals; MNN = mean of normal to normal beat intervals; LF/HF = low-frequency/high-frequency power spectrum; LF(ln) = normalized (natural logarithm) low-frequency power spectrum

Discussion

The PD2i of the heartbeats is believed to predict AD better than the other algorithms for two reasons:

- The data requirements of its underlying mathematical model are met by the physiologic data; ie, the PD2i does not require random data variation or data stationariness, as do the linear and nonlinear mathematical models of the comparator algorithms in **Table 1**
- The heartbeat PD2i measures something relevant to the underlying neurophysiology of heartbeat generation—namely, the degrees of freedom of neural controllers at work at any one moment in time.

The significance of the reduced number of degrees of freedom in the ischemic heart that predicts AD (VF) is not yet fully understood. That the maximum PD2i value in the heart at rest is 6.0 suggests a relationship to the six well-known afferent-efferent loops that compete with one another to control the heartbeats:

- Intrinsic cardiac neurons
- Baroreflex
- Respiratory sinus arrhythmia
- pH modulator
- Temperature controller
- The cerebral defense system (the only loop that receives primary sensory inputs as well as visceral afferents).

These same loops all contribute efferent fibers to the same descending cerebral pathway that has been identified in the pig model of heart attack to regulate the vulnerability of the ischemic heart to VF. That is, the heartbeat is regulated by the same integrated neural system that controls cardiac vulnerability to VF. Thus the heartbeat PD2i is a continuous physiologic measure of the cerebral impact on the heart that controls vulnerability to lethal arrhythmogenesis.

Of special interest is the finding that the PD2i of the R-R intervals in heart transplant recipients is exactly 1.0.[10] This observation is perhaps expected, as only the intrinsic cardiac nervous system remains intact. The mechano- and chemoreceptors located in this most peripheral neural loop enable the heartbeats to increase and decrease in rate (ie, have 1 degree of freedom), but the beat series lacks the more complex jitter caused by the competitive contributions of the other five afferent-efferent loops. Interestingly, at the time the cardiac nerves begin to regenerate back into the transplanted heart, the heartbeat PD2i begins to increase systematically.

The significance of the low-dimensional PD2i excursions (to ≤ 1.4, the separator that best divides the high-risk emergency room patients into those who are and are not at risk of AD) would seem at first

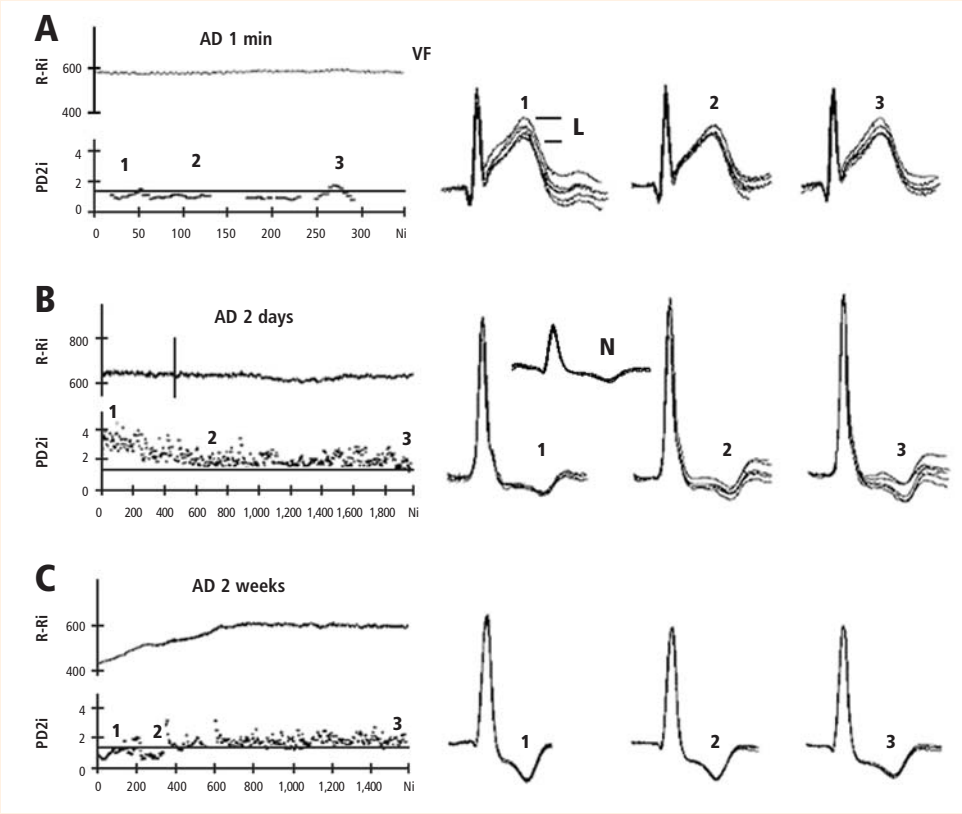

FIGURE 2. Heartbeat intervals (R-Ri), degrees of freedom (PD2i), and T-wave lability (L) in three patients who manifested arrhythmic death (AD) at different times after undergoing electrocardiographic recordings. T-wave lability (L) is indicated by the range of variation of the T waves when five successive heartbeats are superimposed; the superimposed traces were aligned by the PQ intervals preceding each R wave and were sampled at three different locations, indicated by the numbers (1, 2, 3) above the PD2i plots. "N" indicates the trace of a normal patient.

thought to be related to a turning off (inhibition) of many of the afferent-efferent loops. But the low value ($\leq$ 1.4) suggests that there would not be sufficient numbers of vital controllers that remain functional. Furthermore, it does not make sense that 1.4 is a fractional value.

Therefore, a collapse of the orthogonal dimensions of the variations would seem to be a better explanation, as all systems could still participate in heartbeat regulation and actually have a coordinated overall fractional-dimensional effect. So the best interpretation is that cooperation among the previously independent afferent-efferent loops along the brain-heart axis is what best explains the reduced dimensionality. This cooperation is like that of six ensemble musicians who start playing together in harmony instead of in disharmony (resonant cooperativity) or, alternately, like six musicians in an orchestra who follow the conductor (driven cooperativity).

Figure 2 demonstrates what this orchestrated neural harmony descending from the brain does to the heart at risk of VF. As noted above, this figure, which presents findings in three patients who suffered AD at various intervals after their ECG recordings, shows apparent lability in the T waves in two patients who manifested AD either immediately or within a few days. For the patient represented in **Figure 2B**, lability of the T waves did not begin until the PD2i temporally descended to the vicinity of 1.4 (horizontal line), a finding that indicates some relationship between PD2i and the lability of refractoriness. The patient represented in **Figure 2C**, however, did not manifest VF until 2 weeks after the ECG, and in this case there was no T-wave lability, yet there still were PD2i excursions below 1.4 that predicted the later AD. Perhaps the T-wave lability needed more time to develop in this patient's heart and that is why he did not manifest AD more imminently.

Verrier and associates have studied T-wave lability in patients by observing the T-wave difference in alternate beats (T-wave alternans, or TWA). They found that TWA also is a predictor of AD.[11] It would seem, however, that PD2i predicts AD at a time when TWA does not **(Figure 2C)**. This might be because TWA is a measure of a "bad heart" (lability of refractoriness) while PD2i is a measure of a "bad brain" (autonomic cooperativity) and it takes both a bad heart *and* a bad brain to generate the physiologic

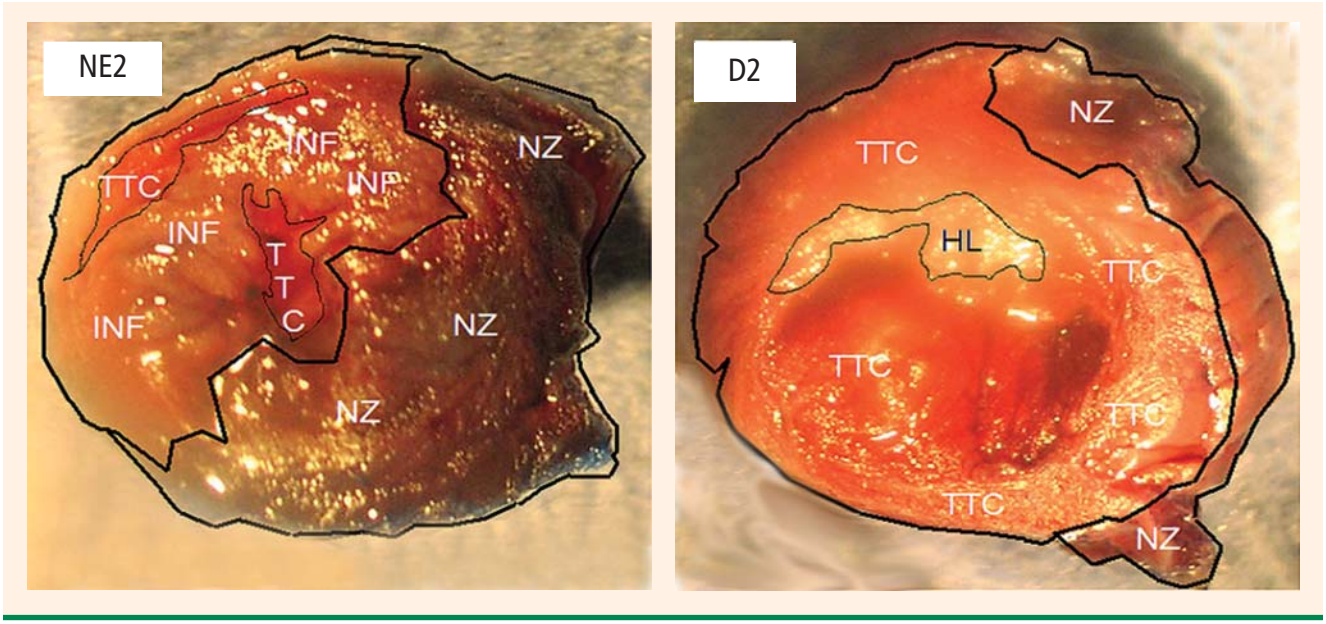

FIGURE 3. Effects of state-dependent molecular fraction with the anti-infarction molecule (D2) on the prevention of myocardial infarction in the rat. The anesthetized and respirated animal underwent 45-minute occlusion of its left anterior descending coronary artery (LAD), after which either the D2 molecule or its control was injected. After 24 hours the heart was sectioned and incubated in triphenyltetrazolium chloride (TTC). **Right panel:** The bright TTC stain indicates normal functioning of heart muscle; highlights are indicated by "HL"; "NZ" indicates nonischemic zones that were perfused during the LAD occlusion and injected later with a blue dye (after retying the LAD). **Left panel:** The control injection (NE2) resulted in unstained infarcted tissue (INF) with islands of TTC staining only at the epicardial and endocardial surfaces.

dynamics underlying initiation of the rotor that Winfree has modeled mathematically[12] and suggested to be the root cause of VF.

Future studies and clinical applications

New clinical studies will first look at the ability of heartbeat PD2i to predict the risk of sudden death in certain high-risk populations. Since only 19% to 21.4% of patients implanted with an automatic defibrillator ever have the device "fire" and shock their hearts out of VF,[13] it would seem that PD2i analysis of individuals referred for such surgery may, as a group, benefit from risk stratification to reduce the number of unnecessary implant surgeries. A large multicenter trial (VITAL) is under way to examine this possibility. Manufacturers of implantable cardioverter defibrillators should appreciate that large-scale and cost-effective screening with the PD2i technology may identify yet more candidates for implantation, as sudden cardiac death remains a major medical problem.

Early application of PD2i analysis is likely to target other high-risk populations. First among these are likely to be patients with ischemic and nonischemic cardiomyopathy, followed by patients with hypertrophic obstructrive cardiomyopathy and hypertensive cardiomyopathy, for which risk stratification is completely lacking. The possibility that such individuals could be successfully identified and directed toward life-saving treatment is exciting. Similarly, patients with the "channel-opathies"—long QT syndrome, Brugada syndrome, etc—would also seem to be good candidates for PD2i analysis.

Eventually, studies will be planned for evaluation of PD2i analysis in large populations at low risk, such as competitive school-aged or professional athletes. Such studies would require an enormous number of (typically young) subjects, but these are the populations in which a rare case of sudden death creates considerable parental concern and media interest. Lastly, studies may be done in individuals who are at moderate risk but may in the future, if the test becomes widely available, simply wish to be screened. Examples would include asymptomatic adults with hypertension or hyperlipidemia.

STATE-DEPENDENT PROTEOMICS

Background and rationale

For our second paradigmatic approach—use of state-dependent proteomics to identify an anti-infarction molecule—a research problem arose because REM sleep is so brief that one cannot expect to sample very many molecules during this period. A better model of REM sleep was sought. Consideration of hibernation

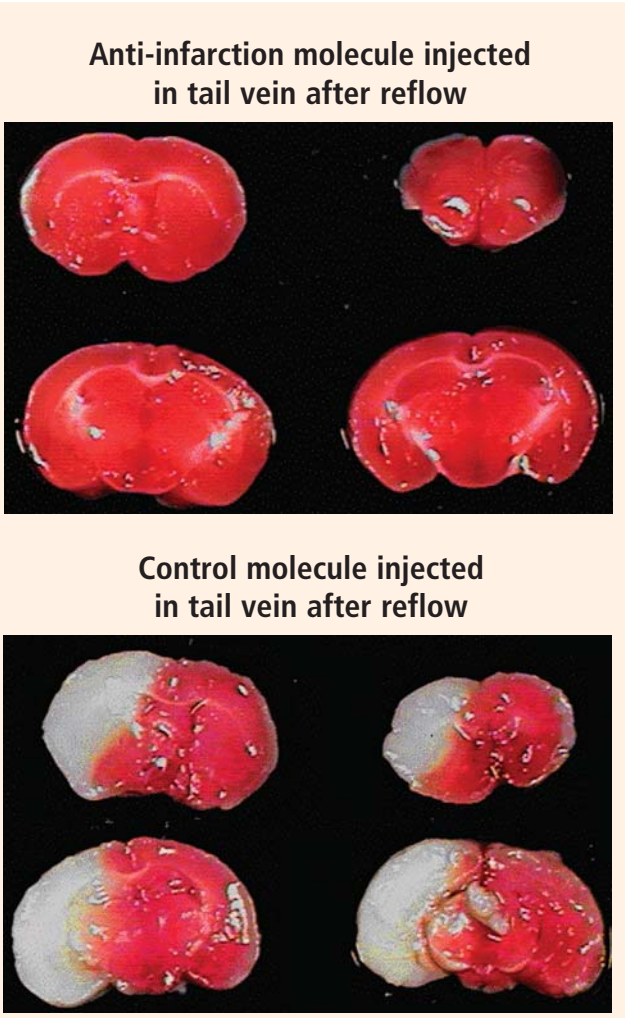

FIGURE 4. Effects on the mouse brain of injection of the isolated anti-infarction molecule (top panel) or its control (bottom panel) following 1-hour occlusion of the middle cerebral artery. Red triphenyltetrazolium chloride (TTC) staining indicates brain tissue that was viable 24 hours after injection. The large white zones in the control mouse indicate a typical cerebral infarction.

was a natural, as it has several of the physiologic features of REM sleep: muscle atonia, autonomic shutdown, and neurosecretion of regulatory proteins and peptides. For these reasons, we chose as our model the hibernating woodchuck; details of the patented state-dependent proteomic methods used to isolate and identify the endogenous molecules in the plasma of hibernating woodchucks are described in another patent (pending).[14]

The early substate of hibernation in the woodchuck was found to have the salutary effect on arrhythmogenesis. To find the proteins and peptides that are expressed in abundance during this substate, high-resolution two-dimensional gels (sodium dodecyl sulfate) and high-performance liquid chromatography were applied to plasma withdrawn during that state and its nearest control state (ie, midhibernation). Differential substate comparisons were used to isolate the salutary molecules. Tandem mass spectrometry fingerprinted the differential upregulated molecules, leading to their identification in worldwide databases. The peptide molecules and their pharmacophores were then synthesized and tested for efficacy in bioassay models.

Methods and results of bioassay models

Coronary artery occlusion in the rat was the first bioassay model. The hibernation-related molecules were indeed found to have a salutary effect on the ischemic heart—they prevented infarctions. An example of this is shown in **Figure 3** for control and experimental molecule subfractions.

The protocol was to occlude the left anterior descending coronary artery for 45 minutes, release the ligature (and electroconvert reperfusion VF, if it occurred), intravenously inject the experimental or control subfraction, and then 24 hours later examine staining of viable tissue in the distal myocardium (the stain triphenyltetrazolium chloride [TTC] is taken up only by functioning mitochondria in viable tissue).

Figure 3 provides a comparison of the widespread bright-red TTC staining in the section of the heart from the rat injected with the salutary molecule (right panel) with the very few such spots in the specimen from the rat injected with the control molecule (left panel).

In the wake of this evidence that the salutary molecule worked in the heart, as expected, it was then quickly tested in another model of ischemia—stroke in the mouse.

The mouse stroke model involved occlusion of the middle cerebral artery for 1 hour. After 1 hour of no blood flow (documented by 0 flow in the parietal cortex by laser Doppler recording), reflow was established (ie, to model the effect of clot-breaking drugs as used in a modern hospital). Immediately after reflow was established, the experimental or control molecule was injected into a tail vein of each of the mice. After recovery, 24 hours later, each animal's behavior was observed for limb paralysis and its brain was extracted and examined for TTC staining. More than 25% of the experimental animals had no histologic or behavioral signs of stroke, whereas severe paralysis and lack of TTC staining occurred in all of the controls.

With the mouse stroke bioassay, we easily isolated

the best peptide molecule, identified it, and then synthesized it. The synthesized molecule and many of its amino acid substrings all worked very well as anti-infarction compounds. With the best pharmacophore, the mean percentage of tissue saved was 77%. An example of a 100% result with that candidate drug is shown in **Figure 4**.

Additional compounds and future studies

Other hibernation-related drugs are still being discovered. For example, pregnant polar bears make proteins for their fetuses during hibernation, but they do not urinate. So what do they do with their blood urea? It was found that they recycle the blood urea back into amino acids.[14] A nonhibernating mammal (the rat) seems to have a small basal level of urea recycling. A hibernation-related molecule was isolated that can stimulate the recycling rate in this nonhibernating animal to a level that approaches the load-handling capability of normal kidney function.[14]

Additional hibernation-related molecules regulate the ongoing physiologies, and these, like the anti-infarction and urea-recycling molecules, may be the basis for discovery of important new drugs. So far, all of the molecules of interest found in hibernating species are also found in nonhibernating species. Regulatory molecules that readily come to mind include anticoagulants, soporifics, antihypertensives, and appetite suppressants, each of which would seem to regulate one or another of the physiologies of hibernation. This plethora of hibernation-related molecules appears to be a gold mine that has been stumbled upon in the quest to unravel the role of the brain in cardiovascular disorders.

CONCLUSION

Successful application of these two new paradigms suggests that heart-brain medicine holds a wealth of possibilities for discovery. The early theories of the pioneer in heart-brain relationships, Walter B. Cannon, have now been supported by neurophysiologic, neurochemical, and neurobehavioral studies, as presented in the preceding article in this supplement. The integration of these results within the framework of Cannon's early ideas about the evolution of the brain, using the new paradigmatic methods of state-dependent proteomics and nonlinear dynamics, presents meaningful new ways of developing devices and drugs in a new era.

REFERENCES

1. **Skinner JE, Carpeggiani C, Landisman CE, Fulton KW.** Correlation dimension of heartbeat intervals is reduced in conscious pigs by myocardial ischemia. Circ Res 1991; 68:966–976.
2. **Skinner JE, Pratt CM, Vybiral T.** A reduction in the correlation dimension of heartbeat intervals precedes imminent ventricular fibrillation in human subjects. Am Heart J 1993; 125:731–743.
3. **Skinner JE, Mohr DN, Kellaway P.** Sleep-stage regulation of ventricular arrhythmias in the unanesthetized pig. Circ Res 1975; 37:342–349.
4. **Skinner JE.** PD2i electrophysiological analyzer. US patent 5 720 294. 1998.
5. **Skinner JE.** PD2i electrophysiological analyzer. US patent 5 709 214. 1998.
6. **Skinner JE.** Method and system for detecting and/or predicting biological anomalies. US patent 0 147 815. 2004.
7. **Lee TH, Juarez G, Cook EF, et al.** Ruling out acute myocardial infarction. A prospective multicenter validation of a 12-hour strategy for patients at low risk. N Engl J Med 1991; 324:1239–1246.
8. **Hinkle LE Jr, Thaler HT.** Clinical classification of cardiac death. Circulation 1982; 65:457–464.
9. **Skinner JE, Meyer M, Nester BA, et al.** Risk stratification for arrhythmic death in an emergency department cohort: a new method of nonlinear analysis of RR-intervals. Submitted to Ann Emerg Med; 2006.
10. **Meyer M, Marconi C, Ferretti G, Fiocchi R, Cerretelli P, Skinner JE.** Heart rate variability in the human transplanted heart: nonlinear dynamics and QT vs RR-QT alterations during exercise suggest a return of neurocardiac regulation in long-term recovery. Integr Physiol Behav Sci 1996; 31:289–305.
11. **Nearing BD, Verrier RL.** Analysis of complex T-wave oscillations for prediction of ventricular fibrillation. J Electrocardiol 2003; 36(Suppl):199–203.
12. **Winfree AT.** Electrical instability in cardiac muscle: phase singularities and rotors. J Theor Biol 1989; 138:353–405.
13. Centers for Medicare and Medicaid. Proposed decision memo for implantable defibrillators (CAG-00157R3), 2005.
14. **Skinner JE, Anchin JM.** Anti-infarction molecules. US patent 0 228 371. 2003.

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