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The Effect of Cardiac Arrest on the Blood-Testis Barrier to Albumin, Tumor Necrosis Factor-Alpha, Pituitary Adenylate Cyclase Activating Polypeptide, Sucrose, and Verapamil in the Mouse

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ABSTRACT: Impotence commonly occurs after events such as acute myocardial infarction, coronary bypass, head trauma, and cerebral bleeding, including subarachnoid hemorrhage. We hypothesize that the hypoxia accompanying these events could damage the blood-testis barrier (BTB) and so cause testicular dysfunction, a possible cause of impotence. We examined the effect of cardiac arrest in mice on testis weight and various aspects of BTB function. Testis weight was decreased by about 24% 12 hours after cardiac arrest but had recovered fully by day 7. The testis/serum ratio for albumin was increased 12 hours after arrest, showing a disruption in the vascular BTB with recovery by 24 hours. The testis/serum ratio for sucrose was not consistently elevated, showing that the Sertoli cell BTB remained intact. The testis/serum ratio for verapamil was increased on day 3 of cardiac arrest, suggesting impaired function of the BTB's p-glycoprotein efflux transporter. Transporters for pituitary adenylate cyclase activating polypeptide and tumor necrosis factor-α were not affected by cardiac arrest. These results show that cardiac arrest affects testis weight and some aspects of BTB function. Such changes might have long-term effects on testicular function.

Key words: Ischemia, cardiac arrest model, testis, impotence.
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Impotence can occur after coronary and cerebrovascular events. In particular, myocardial infarction, cardiac bypass, cardiac arrest, and subarachnoid hemorrhage (Takaku et al, 1979) are associated with the onset of impotence. The cause of impotence after these events is still unclear but may be due to the associated hypoxia. Clinical experience has long linked hypoxia with impotence. Numerous pathways have been suggested that could mediate hypoxia-related impotence, including altered hormonal levels due to testicular or hypothalamic dysfunction (Semple et al, 1984a, 1984b; Banks et al, 1990; Mills et al, 1992). In addition, the production of nitric oxide, which is important in penile erection (Burnen et al, 1993; Pickard et al, 1995), is impaired after cardiac arrest (Stepanichev et al, 1998). A key player in the hormonal regulation of the testis and to nitric oxide production is the blood-testis barrier (BTB). Dysfunction of the BTB could occur after hypoxia, as it does for the blood-brain barrier (Mizushima et al, 1999a, 1999b). Dysfunction of the BTB could lead to testicular dysfunction, which in turn, could be a contributing factor to impotence.

The BTB consists of several barriers in series that divide the testis into compartments (Neaves, 1997). The vascular barrier and the Sertoli cell barrier are its 2 major components, interfacing between the blood and the testicular interstitial fluid and between the testicular interstitial fluid and the seminiferous tubule fluid, respectively.

We studied the function of these barriers after cardiac arrest with a model we developed for mice (Dohi et al, 1998; Korpatchev et al, 1998; Mizushima et al, 1999a,b). This model is very similar to clinical scenarios with resuscitation after a few minutes of hypoxia secondary to cardiac arrest. We used this model to examine the effects of cardiac arrest on the integrity of the vascular BTB as measured with radioactively labeled albumin and the integrity of the Sertoli cell BTB as measured with radioactive sucrose. The transport across the BTB of 3 substances likely to be important to testicular function after hypoxia was also examined. Pituitary adenylate cyclase

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activating polypeptide (PACAP) is important in sperm maturation (Shioda et al, 1998), has a saturable transporter located at the vascular BTB (Banks et al, 1993a), and is known to inhibit hypoxia-induced cell death (Uchida et al, 1996). Tumor necrosis factor-α (TNF) has roles in testicular function (Mealy et al, 1990; Meikle et al, 1992; Le Magueresse-Battistoni et al, 1997), in mediating hypoxic and immune damage (Pan et al, 1997), and is released after cardiac arrest (Colletti et al, 1990). The p-glycoprotein transporter, measured here with radioactive verapamil, is an efflux system, which likely rids the testis of toxins (Holash et al, 1993).

Material and Methods

Animals

Adult male ICR mice (10–12 weeks, 30–35 g; Charles River Labs, Inc, Wilmington, Mass) were housed in a temperature- and light-controlled room (lighting from 0600 to 1800 hours) with standard laboratory chow and water ad libitum. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Showa University.

Induction of Cardiac Arrest and Resuscitation

Mice were placed in a plastic box filled with halothane gas for about 20 seconds and, after the onset of anesthesia, were connected to an anesthesia mask for mice. Anesthesia was maintained with 3.5% isoflurane in 40% N_2O and 15% O_2. Body temperature was maintained at 37°C. Global ischemia was induced according to the cardiac arrest model (Korpatchev et al, 1998). The anesthetized mouse was placed in a supine position and the upper extremities were fastened to a board in the extended position with pins. An incision about 2.0 cm long was made in the midline of the chest up to the maxillary gland. An L-shaped occluding device was gently inserted in the mediastinum at the level of the fourth intercostal segment with its distal end at the dorsal wall of the thorax. The distal end was twisted 45 degrees to position it under the bundle of major cardiac blood vessels. A complete interruption of cardiac output caused by compression on the major cardiac vessels was accomplished by lifting upward the occluding device and, at the same time, applying modest downward pressure with the fingers on the sternum. After 3 minutes of arrest, the mouse was resuscitated with external heart massage while ventilating with a tidal volume of 2.5 mL at 170 times per minute (4 L/min O_2). Heart massage was continued until spontaneous heart contractions began (about 3 minutes) and ventilation continued for about 20 minutes after which the mouse was disconnected from the respirator and housed in a cage with free access to water and food pellets. Control mice were anesthetized with halothane and given isoflurane for 5 minutes.

Radioactive Labeling of Albumin, TNF-α, PACAP, Sucrose, and Verapamil

Albumin was labeled with ^{99m}Tc (Tc-albumin) with the kit from Medi-Physics (Paramus, NJ). Five microliters of recombinant murine TNF-α (R & D Systems, Minneapolis, Minn) was labeled with ^{131}I (I-TNF) by the enzymobead method (Bio-Rad, Richmond, Calif). The I-TNF was purified on a column of Sephadex G-10 by elution with 0.25 M chloride-free phosphate-buffer solution and had a specific activity of about 40 mCi/mg. Synthetic PACAP 38 (Sigma Chemical Company, St Louis, Mo) was labeled with ^{131}I obtained from New England Nuclear, Inc (Boston, Mass) by the lactoperoxidase method and purified on a column of G10 Sephadex. Specific activity was about 500 mCi/mg, as previously assessed by the self-displacement method of immunoassay (Gottschall et al, 1990). ^{14}C-sucrose (C-sucrose; 550 mCi/mMol) and ^{3}H-verapamil (H-verapamil; 84 Ci/mMol) were purchased from New England Nuclear.

Measurement of Blood-Testis Barrier Permeability

Mice were studied 6 or 12 hours after cardiac arrest (day 1 is day of arrest) or on day 2 (24 hours postarrest), 3, 5, or 7. Under intraperitoneal anesthesia with 40% urethane, the left jugular vein and right carotid artery were isolated and 0.2 mL of lactated Ringers solution with 1% bovine serum albumin injected into the left jugular vein (intravenous [IV] injection). The injection solution contained about 1 000 000 cpm (gamma emmitters) or dpm (beta emmitters) of Tc-Alb, C-sucrose, I-PACAP, I-TNF, or H-verapamil. Ten minutes after the IV injection, blood was collected from a cut in the right carotid artery to obtain serum, and was followed by immediate decapitation. The testes were removed, weighed, and the levels of radioactivity in the testis and in the serum measured in a gamma counter (I-TNF, Tc-Alb, and I-PACAP) or a beta counter (C-sucrose and H-verapamil) for 10 minutes.

Statistical Analysis

The results were expressed as testis/serum ratios in units of μL/g: (cpm/g of testis)/(cpm/μL of serum) = μL/g. Means are reported with their standard errors. Group means were compared by analysis of variance (ANOVA) followed by Newman-Keuls multiple range test when more than 2 means were compared.

Results

Cardiac arrest had statistically significant effects on testicular weight: $f(6, 70) = 3.42$, $P < .01$. Figure 1 shows the time-dependent changes in testicular weight (n = 10–11/group). The range test showed that testicular weight was lower 12 hours after arrest ($P < .01$) in comparison to the control mice. The day 7 value was also statistically different from the 12-hour value ($P < .01$) but not from the control mice, demonstrating full recovery of testicular weight by day 7.

Figure 2 shows the effect of cardiac arrest on the permeability of the BTB to albumin. ANOVA showed a significant effect: $f(6, 20) = 4.55$, $P < .005$, and the range test showed that permeability was increased 12 hours after arrest in comparison to controls ($P < .01$), 6-hour ($P < .05$), 24-hour ($P < .01$), 3-day ($P < .01$), 5-day ($P <$

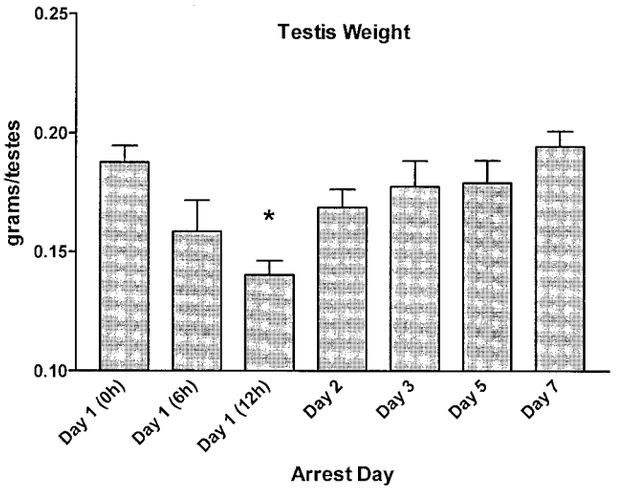

Figure 1. Effects of cardiac arrest on testis weight. The Y axis gives the weights for both testes combined and the X axis shows time after arrest. The day of arrest was counted as Day 1, and Day 1 (0 hour) represents control mice (not arrested). *Twelve hours postarrest weights were significantly different from those for control mice ($P < .01$).

.05), and 7-day ($P < .05$) values (n = 3–4/group). A repeat of this experiment produced almost identical results.

Figure 3 shows the effect of cardiac arrest on the permeability of the BTB to sucrose. ANOVA showed a significant effect: f(6, 40) = 2.57, $P < .05$. However, the range test showed no significant differences between controls and any postarrest values (n = 6–7/group). There

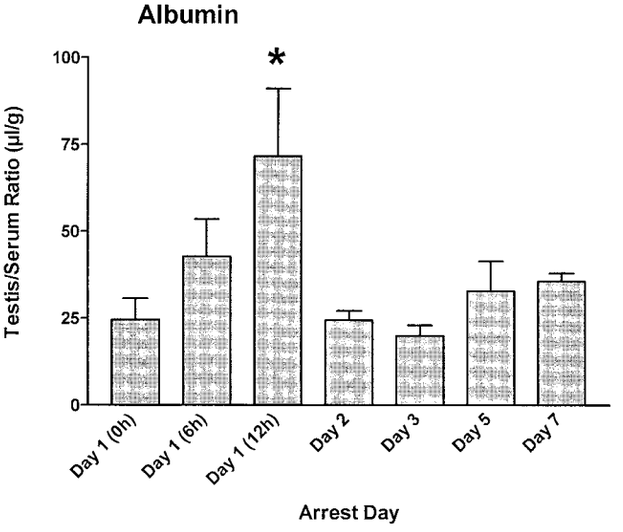

Figure 2. Testis/serum ratio for albumin after cardiac arrest. The Y axis measures the amount of radioactively labeled albumin in the testis after its IV injection and the X axis shows time after arrest. The day of arrest was counted as Day 1 and Day 1 (0 hour) represents control mice (not arrested). Albumin measures the integrity of the vascular barrier. *Twelve hours postarrest values showed a statistically significant increase ($P < .01$).

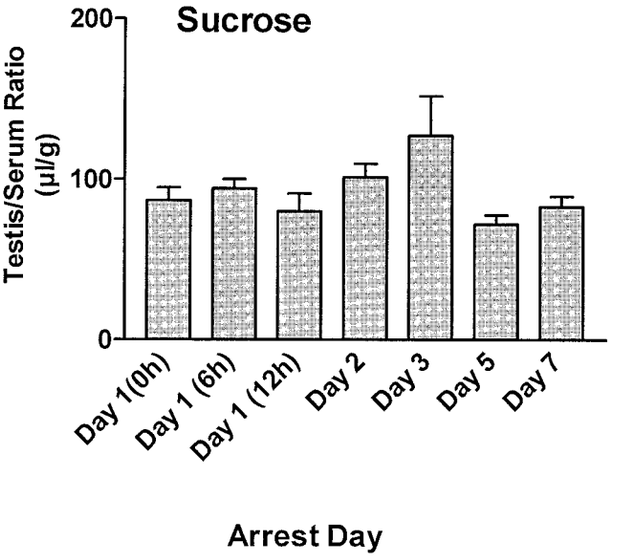

Figure 3. Testis/serum ratio for sucrose after cardiac arrest. The Y axis measures the amount of radioactively labeled sucrose in the testis after its IV injection and the X axis shows time after arrest. The day of arrest was counted as Day 1 and Day 1 (0 hour) represents control mice (not arrested). Sucrose measures the integrity of the Sertoli cell barrier. Day 3 postarrest values showed a trend ($P = .09$) toward a significant difference when compared with controls (Day 1, 0 hour) values.

was a trend ($P = .09$) toward a difference between the control and day 3 values.

Figure 4 shows the effect of cardiac arrest on the permeability of the BTB to verapamil. ANOVA showed a significant effect: f(6, 11) = 10.9, $P < .0005$. The range test showed that permeability was increased on day 3 (48 hours after arrest) after arrest in comparison to controls

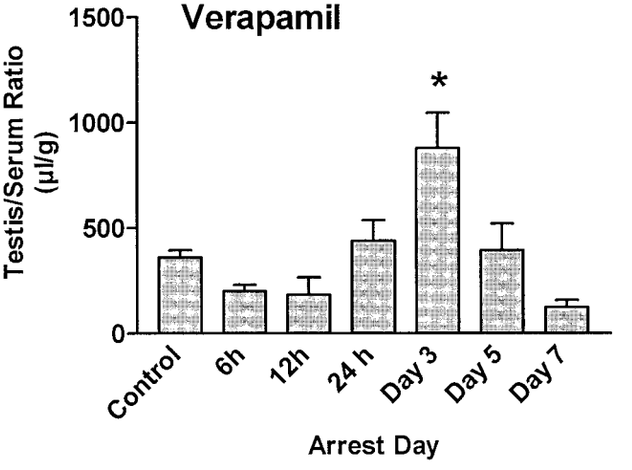

Figure 4. Testis/serum ratio for verapamil after cardiac arrest. The Y axis measures the amount of radioactively labeled verapamil in the testis after its IV injection and the X axis shows time after arrest. The day of arrest was counted as Day 1 and Day 1 (0 hour) represents control mice (not arrested). Day 3 postarrest values showed a statistically significant increase ($P < .01$).

($P < .01$), 6-hour ($P < .001$), 24-hour ($P < .01$), 5-day ($P < .01$), and 7-day ($P < .001$) values (2–3/group).

Cardiac arrest had no statistically significant effect on the permeability of the BTB to TNF (Figure 5A) or PACAP (Figure 5B).

Discussion

The differential diagnosis for impotence has changed dramatically over the last 30 years, with a shift from the assumption that most cases are psychogenic to a demonstration that most cases have an organic cause. Penile blood pressure measurements, nocturnal penile tumescence (NPT) studies, and studies of the arterial bed supplying the cavernous bodies have shown that the majority of cases are vascular (Michal, 1982). Arterial disease, a very common affliction of the middle-aged population, is largely implicated in the pathogenesis and etiology of impotence (Michal, 1982; Morley et al, 1988). Whereas the above illustrates the importance of the local vascular supply for proper function, hypoxia from systemic causes has also been implicated (Semple et al, 1984a, 1984b; Karacan et al, 1995). Systemic hypoxia can affect the entire hypothalamic-gonadal axis. The model of cardiac arrest used here is very similar to the global hypoxia induced by cardiac arrest in the clinical setting and is associated with loss of hippocampal neurons (Dohi et al, 1998) and changes in the permeability of another tissue barrier, the blood-brain barrier (Dohi et al, 1998; Korpachev et al, 1998).

Here, we investigated the effect of cardiac arrest on the BTB by using radioactive substances that measure various aspects of BTB function: albumin, sucrose, verapamil, TNF, and PACAP.

Albumin is a 60-kilodalton (kd) protein with a passage that is restricted by the vascular BTB. Any albumin that does enter the testicular interstitial fluid space is further restricted by the Sertoli cell barrier. We found here that 6 hours after cardiac arrest, disruption of the vascular BTB as measured with albumin was accompanied by a decline in testicular weight. A fluid shift out of the testis made possible by the disruption of the vascular BTB or a reduction in spermatogenesis could cause such a sudden, reversible change in weight. Although the changes in weight and in barrier permeability were rapidly reversed, the effects on testicular function may be longer lasting. By analogy, testicular failure can occur years after acute viral orchitis and even in the contralateral testis. The acute changes seen here could set in motion a series of events that could eventually lead to testicular dysfunction and impotence. Sucrose readily penetrates the vascular BTB but is restricted from entering the seminiferous tubule barrier by the Sertoli cell BTB (Fritz et al, 1983). ANOVA showed statistical differences in the uptake of sucrose by the testis after cardiac arrest, but Tukey's post-test found no statistical differences among the groups. We interpret this to mean that disruption of the Sertoli cell BTB was

Figure 5A

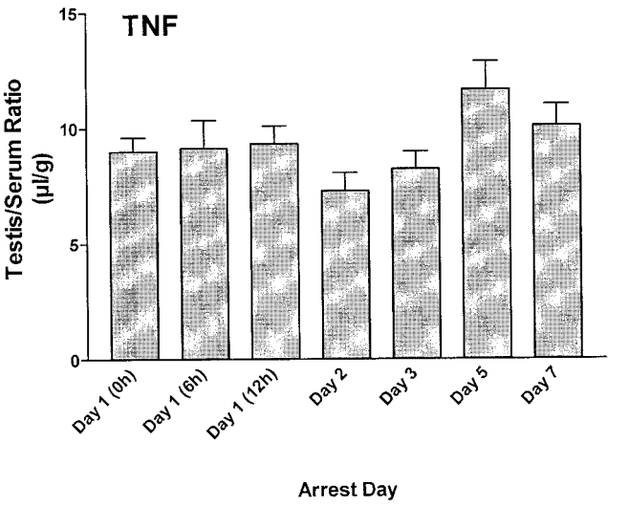

Figure 5B

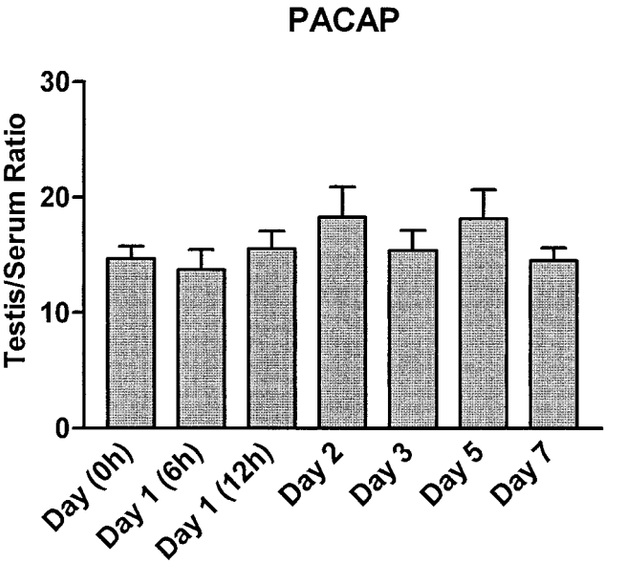

Figure 5. Testis/serum ratio for TNF-α and PACAP after cardiac arrest. **(A)** TNF; **(B)**PACAP. The Y axes measure the amount of radioactively labeled TNF or of radioactively labeled PACAP in the testis after their IV injection and the X axis shows time after arrest. The day of arrest was counted as Day 1 and Day 1 (0 hour) represents control mice (not arrested). There were no significant differences from controls for either TNF or PACAP.

minimal if it occurred at all. Therefore, the vascular barrier is much more sensitive to hypoxic changes than is the Sertoli cell barrier.

Restriction of blood-borne substances is only one aspect of the function of the BTB. Because the vascular BTB limits the production of an ultrafiltrate, nutrients, and other substances required by the testis are transported in by specific, saturable systems and toxins are transported out.

An example of one such specific, saturable transport system is the one for PACAP, which is transported across the blood-brain barrier, the blood–spinal cord barrier (Banks et al, 1993a, 1998), and the BTB (Banks et al, 1993). PACAP is a member of the secretin/glucagon/vasoactive intestinal peptide (VIP) family (Arimura, 1992). PACAP is found in high levels in the testis where it plays an important role in the maturation of sperm (Shioda et al, 1998). PACAP has potent neurotrophic and neuroprotective effects (Deutsch et al, 1992, 1993; DiCicco-Bloom and Deutsch, 1992), including protection against hypoxia (Uchida et al, 1996). The PACAP transporter located at the blood-brain barrier is altered by hypoxia induced by the cardiac arrest model used here (Mizushima et al, 1999). In comparison, we found that hypoxia did not alter the permeability of the BTB to PACAP.

TNF is an endogenous cytokine produced in both the central nervous system (CNS) and the peripheral tissues, which is elevated in the early phase of CNS trauma and inflammation. TNF has effects on testicular function (Mealy et al, 1990; Meikle et al, 1992; Le Magueresse-Battistoni et al, 1997), interacts with endothelial cells (Barten et al, 1994; Deli et al, 1995; Estrada et al, 1995; Pan et al, 1997), and is associated with a decrease in plasma levels of testosterone and increases in plasma levels of luteinizing hormone and follicle-stimulating hormone (Mealy et al, 1990). The 17-kd monomer of TNF circulates in its active form as a homotrimer that is close in size to albumin. Unlike albumin, TNF is transported into the CNS by a specific, saturable transport system (Gutierrez et al, 1993). Because TNF (Smith et al, 1987; Wingfield et al, 1987) is degraded in the circulation and is more rapidly transported across barriers than albumin, a shorter time interval was chosen for study. Similar experimental protocols have shown that TNF transport into the brain is affected by various types of CNS injury (Pan et al, 1996, 1997) but not by cardiac arrest (Mizushima et al, 1999). We found here that TNF uptake by the testis was not affected by cardiac arrest.

Verapamil is a substrate for the p-glycoprotein efflux system. This system is located at various barriers, including the BTB (Holash et al, 1993). We found that the testis/blood ratio for verapamil increased on postarrest day 3 (about 48 hours after arrest). Because this is an efflux system, an elevation in the testis/serum ratio means that efflux is impaired. Loss of p-glycoprotein function at the blood-brain barrier has been shown to result in increased neurotoxicity and central nervous system actions of those substances transported by this system (Schinkel et al, 1996). Impairment of p-glycoprotein at the BTB means that the testis is much more vulnerable to a large number of blood-borne substances. Many of the substrates of p-glycoprotein are drugs to which postarrest patients might be exposed.

In conclusion, the results show that after cardiac arrest, some of the functions of the BTB are altered, including the integrity of the vascular barrier and the activity of the p-glycoprotein efflux system, whereas other functions are not. Changes do not all occur at the same time, with vascular disruption peaking 6 hours after cardiac arrest and p-glycoprotein dysfunction peaking 48 hours after arrest. It is likely that other functions of the BTB are altered with cardiac arrest. These changes in BTB function might underlie the eventual development of testicular dysfunction seen after cardiac arrest and other causes of systemic hypoxia.

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