Alterations in pulmonary surfactant after rapid arousal from torpor in the marsupial *Sminthopsis crassicaudata*

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1Department of Physiology, The University of Adelaide, Adelaide, South Australia 5005; and 2Respiratory Research Group, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Lopatko, Olga V., Sandra Orgeig, David Palmer, Samuel Schürch, and Christopher B. Daniels. Alterations in pulmonary surfactant after rapid arousal from torpor in the marsupial *Sminthopsis crassicaudata*. J. Appl. Physiol. 86(6): 1959–1970, 1999.—Torpor in the dunnart, *Sminthopsis crassicaudata*, alters surfactant lipid composition and surface activity. Here we investigated changes in surfactant composition and surface activity over 1 h after rapid arousal from torpor (15–30°C at 1°C/min). We measured total phospholipid (PL), disaturated PL (DSP), and cholesterol (Chol) content of surfactant lavage and surface activity (measured at both 15 and 37°C in the captive bubble surfactometer). Immediately after arousal, Chol decreased (from 4.1 ± 0.05 to 2.8 ± 0.3 mg/g dry lung) and reached warm-active levels by 60 min after arousal. The Chol/DSP and Chol/PL ratios both decreased to warm-active levels 5 min after arousal because PL, DSP, and the DSP/PL ratio remained elevated over the 60 min after arousal. Minimal surface tension and film compressibility at 17 mN/m at 37°C both decreased 5 min after arousal, correlating with rapid changes in surfactant Chol. Therefore, changes in lipids matched changes in surface activity during the postarousal period.

Pulmonary surfactant is crucial for maintaining lung compliance, preventing lung collapse and pulmonary edema, and stabilizing the gas-exchange area of the lung on expiration. Lowering surface tension will alter alveolar architecture to maintain optimal area available for gas exchange. The ability to adsorb at the air-liquid interface and to lower and vary surface tension during a cycle of alveolar expansion and compression is termed surface activity, a property of surfactant that is strongly dependent on its composition. Surfactant is a complex mixture of lipids and proteins that is synthesized and stored in alveolar type II cells and secreted into the alveolar fluid layer. One of the disaturated phospholipids (DSP) of pulmonary surfactant, dipalmitoylphosphatidylcholine (DPPC), is the major surface tension-lowering agent. Unsaturated phospholipids (USP) and the neutral lipid, cholesterol (Chol), are thought both to promote the adsorption of lipids to form a surfactant film at the air-liquid interface and to aid in the respreading of the molecules after compression (9, 18). The specific surfactant-associated proteins are also capable of modifying the biophysical properties of surfactant because they significantly influence the lipid adsorption and the film formation (22).

Temperature affects the physical state of lipids and therefore influences the surface active properties and, possibly, the functional capacity of surfactant. Surfactant lipids are relatively fluid at 37°C but can be converted into the gel state by either the selective exclusion of lipids with lower melting points or by decreasing temperature. The ability of surfactant lipids to adsorb and respread at the air-liquid interface is significantly reduced at temperatures lower than their melting points (12). Studies on pulmonary surfactant in ectotherms have demonstrated that lipid composition changes with either increasing or decreasing temperature, which represents a typical “homeoviscous” response associated with membranes under different temperature regimes (12). Cold-acclimated desert lizards and map turtles increase the amount of fluidizing agents, such as Chol and/or USP, thereby maintaining optimal surfactant fluidity at low body temperature (4, 14). Turtles acclimated at higher temperatures increase the amount of saturated fatty acids and phosphatidylcholine (PC) in their surfactant (14). It appears, therefore, that there must be a delicate balance in the lipid composition, associated with temperature fluctuations so that surfactant can always achieve rapid adsorption and demonstrate low surface tension on compression at any temperature (12).

The heterothermic mammal, the fat-tailed dunnart, *Sminthopsis crassicaudata*, regularly experiences large, 15–20°C fluctuations in body temperature when it enters and arouses from daily torpor. Torpor is a short-term (maximum of 19 h) reduction in body temperature and metabolic rate by endotherms, which enables energy conservation during periods of reduced ambient temperature and/or low food availability (7, 16). Recently, we determined that, after 8 h of torpor in the dunnart, there was a major increase in the amount of both total phospholipid (PL) and Chol in the whole lung lavage fluid. Concomitantly, there was an alteration in the composition of the lipid with an increase in Chol/PL, Chol/DSP, and DSP/PL ratios (13). These changes in surfactant lipid composition also correlate with changes in surface activity measured in vitro, both in the Wilhelmy-Langmuir balance (WLB) and in the captive bubble surfactometer (CBS) (15). We found that surfactant from torpid dunnarts had a greater surface tension-lowering ability when measured at 20°C than surfactant from warm-active animals. These findings

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suggest that the alterations in surfactant composition and activity during torpor represent an adaptation to the greatly reduced body temperatures. However, the compositional alterations during torpor appear to render the surfactant less functional at 37°C (15).

To our knowledge no study has demonstrated how rapidly altered surfactant composition can return to normal. The arousal from torpor in S. crassicaudata can be very rapid, with the body temperature increasing from 10–15 to 32–35°C at a rate of 0.7–1°C/min (8). The purpose of this study was to investigate the effect of rapid arousal from torpor on surfactant composition and surface activity in S. crassicaudata. Here, we demonstrate that 1) surfactant lipid composition undergoes rapid changes during arousal from torpor; 2) these compositional changes correspond with changes in surface activity; and 3) the changes in surfactant lipid composition and surface activity occur over a period of 1 h after arousal from torpor.

### MATERIALS AND METHODS

#### Animals and Experimental Groups

Male S. crassicaudata (mass range: 10–15 g, n = 48) were purchased from a colony at the University of Adelaide, Australia. Animals were housed individually in cages of 15 × 28 cm and subjected to a 16:8-h light-dark cycle, which commenced at 10:30 PM. Ambient temperature was maintained at 24°C. They were fed ad libitum a mixture of dried canned commercial pet food. The animals were randomly assigned to six experimental groups (see below).

The initial rectal temperature was measured with a thermometer (9-V FLUKE digital thermometer, Everett, WA) to confirm a body temperature over 30°C. In this condition, S. crassicaudata are active and alert. They were termed “warm-active.” To induce torpor, food was removed from the dunnart cages at 5:00 PM on the day before experimentation, and cages were placed in a constant-temperature cabinet (modified Kelvinator) maintained at 24°C. The following morning at 7:00 AM the ambient temperature was reduced to 10°C. Animals were checked at 9:00 AM, and body temperature was recorded. Animals usually take 30–90 min to enter torpor (13, 15). Animals that did not enter torpor were removed from the cabinet and fed, to be used on another day. Torpid animals were identified by a characteristic body posture, which included either lying flat with splayed legs or curled tightly into a ball. Animals with a body temperature lower than 20°C were defined as torpid. The torpid state was maintained for 8 h. Torpid animals were checked frequently to ascertain that they did not rouse from torpor during this time. Six experimental groups (n = 7–9 animals each) included a group of warm-active dunnarts, a group of torpid dunnarts killed 8 h after the onset of torpor, and four groups of animals killed immediately (0 min) or 5, 30, or 60 min after arousal from torpor. To initiate rapid arousal from torpor, we held animals in our hands and frequently checked their body temperature. Body temperature reached a plateau value of 30 ± 0.1°C after 15 min. Warmed-up animals were active and able to maintain the body temperature of 30.6 ± 0.2°C when left in the cage at 24°C. Body temperature was frequently checked and recorded before sampling.

#### Lavage Procedure

Animals were killed quickly with an intraperitoneal overdose of pentobarbitone sodium (~600 mg/kg; Boehringer Ingelheim). The trachea was exposed and cannulated. The cannula was ligated tightly to prevent leakage and connected to a syringe. Lungs were ventilated manually several times with a tidal volume of ~0.8 ml (total lung capacity = 1.0 ml) and then lavaged with 0.15 M NaCl. Three consecutive volumes (0.85 ± 0.05 ml) were infused and withdrawn three times from a syringe filled with 2 ml of saline (15). All lavage material was briefly stored on ice and then centrifuged at 150 g for 5 min (Beckman model T-6) to remove macrophages and cellular debris. Of the total lavage volume (6 ml) obtained from each animal, 3 ml were used for lipid extraction and the determination of total PL, DSP, and Chol contents. The remaining supernatant was lyophilized and stored at −70°C before the surface activity measurements. Lipids were extracted with chloroform-methanol (1:2) by using the method of Bligh and Dyer (2). Total PL was determined by using our standard protocol, by multiplying the measured amount of inorganic phosphorus by 25, because PL is composed of ~4% phosphorus (13, 15). PL amounts were expressed in milligrams per gram dry lung (DL). All the chemicals used in these methods were purchased from BDH Analar and were of an analytic grade.

#### Extraction of DSP and Neutral Lipids

DSP and neutral lipids were extracted by using absorption column chromatography (13). After reaction with osmium tetroxide, the sample was applied to a column of neutral aluminium oxide. Neutral lipids were eluted first with 10 ml of chloroform-methanol (20:1) and stored for later Chol analysis. DSP were eluted with 5 ml of chloroform-methanol-7 M aqueous ammonia (70:30:2). The eluant was evaporated under nitrogen and analyzed for phosphorus content. All the chemicals were purchased from BDH Analar and were of an analytic grade. The amount of DSP is expressed as a percentage of total PL. The denominator (i.e., total PL) in DSP/total PL and in Chol,total PL ratios was determined by phosphorus analysis of the total lipid extract.

#### Chol Analysis

Chol content of the neutral lipid fraction was measured by using a HPLC system composed of an LKB 2157 autosampler (Pharmacia Biotechnology, Uppsala, Sweden) and a Waters pumping system (model M-45; Waters, Milford, MA) (3). Either standard or sample (20 µl each) was injected into a Waters 150°C Novopak guard and analytic column (150 × 4.6 mm I.D) packed with 4-µm spherical silica. Isocratic elution of Chol was completed within 32 min at room temperature by using a mobile phase composed of acetonitrile-190, isopropanol, and water (6:3:3). All the solvents were HPLC grade. The flow rate was maintained at 1 ml/min, and the operating pressure at 1,600 psi. Ultraviolet absorbance was monitored at 210 nm in a model 481 Lambda Max LC spectrophotometer (Waters). The detector output was digitized by using a Delta chromatography data system for acquisition of data and integration of peaks (Digital Solutions, Brisbane, Australia). Standards were assayed in duplicate and included at the beginning of each run (linear from 0.3 to 50 µg/ml, r > 0.995).

#### Surface Activity Measurements

A CBS was used for surface activity measurements at 15 and 37°C. The design of the CBS has been previously described (26). Before the surface activity measurements were made, freeze-dried surfactant samples (containing a total of 387 ± 20 µg PL) were reconstituted to a final lipid concentration of 1 mg/ml, resulting in 1.36 ± 0.08 M NaCl. Because this salt concentration is an order of magnitude
greater than physiological, we performed a series of pilot experiments by using surfactant from warm-active and 8-h-torpor groups as well as bovine lipid extract surfactant with normal or high salt concentrations equivalent to those used in the present study. We found no effect of increasing salt concentrations on the surface activity (adsorption rate, minimal surface tension \([ST_{min}]\), and compressibility) of surfactant samples. In other pilot experiments, the excess salt was washed out of the subphase in the CBS after bubble formation, and the subphase was replaced by 0.15 M NaCl containing 1.5 mM CaCl\(_2\) (pH 6.9) by using the method of subphase depletion (24). The results of these experiments confirmed that a high salt concentration does not significantly influence surfactant surface activity. Where possible we measured the surface activity of individual samples. Where the amount of PL was not sufficient, two, or in some cases three, samples were combined to give a standard concentration of PL for surface activity measurements. Samples were divided into two volumes to study the surface activity at both temperatures. The sample was placed into a small 200-µl sample chamber of the CBS, cooled down to 15°C or warmed up to 37°C, respectively, for 20–25 min while being stirred continuously. Measurements were performed in duplicate or, where possible, in triplicate, by using independent aliquots of the sample at each temperature. We found very small variations in parameters of surface activity both between combined samples within each group and between triplicates.

Adsorption

After 5 min of incubation at a designated temperature, an air bubble with a diameter of 2–3 mm was introduced into the chamber. The moment the bubble assumed its resting shape was considered as “0 min” and indicated the initial adsorption. The bubble was continuously monitored for 10 min by using a video system, and the surface tension, area, and volume of the bubble were calculated from digitized images (25).

Quasi-Static Compression-Expansion Cycles

After the initial adsorption for 10 min, the bubble was compressed stepwise until it reached \(ST_{min}\) (that point where further compression does not result in a further reduction in surface tension) and then expanded stepwise to the original volume. Each semicycle lasted ~1–2 min, depending on the \(ST_{min}\) that could be reached, and the surfactant film was allowed to stabilize for ~5 s after each step. We performed five compression-expansion cycles on each bubble, with 5 min of equilibration time between cycles. The bubble was monitored continuously. Surface tension-area isotherms were generated for the first, second, and fourth cycles, and film compressibility at 17 mN/m \((C_{17})\) (28) was calculated according to the equation \(C_{17} = (1/A)(da/da)\), where \(A\) is the area at a surface tension of 17 mN/m relative to the area at the beginning of the compression; \(a\) is the surface tension; and \(da/da\) is the slope of the tangent to the curve at the surface tension of 17 mN/m. \(ST_{min}\) and \(C_{17}\) of the four compression-expansion cycle are presented (see Figs. 4 and 5). Representative surface tension-area isotherms (4th compression-expansion cycle) at 37°C for surfactant from warm-active, 8-h torpor, 0 min after arousal, and 60 min after arousal groups (means of 2–3 animals) are given (see Fig. 6).

Statistical Analyses

Statistical analyses of body mass, body temperature, and surfactant lipid composition data (absolute and relative amounts of total PL, DSP, and Chol) were performed by using a one-way analysis of variance followed by post hoc t-test, and results are expressed as means ± SE. Ratios were arcsine transformed before analysis.

One of the purposes of this study was to demonstrate the relationship between surface activity and the time after arousal. Because samples from six to seven animals were often combined to give two to three measurements, we did not perform the conventional statistics for between-group comparisons. Instead, we presented the data as means of six to seven animals and chose the “best fit” mathematical function to describe the relationship between surface activity and the time after arousal. The changes in the surface activity and in the lipid composition of surfactant that occurred over the 60-min period (at 0-, 5-, 30-, and 60-min time points) after rapid warming up were described by either linear or logarithmic functions \((y = a + bx\) or \(y = ax^b\), respectively) fitted to the data. The correlation coefficient \((r^2)\) was used to determine the corresponding P value (from statistical Table 8 of Yamane (30)). These values are presented for each curve and indicate whether the changes that occurred in the lipid composition and surface activity during the time after arousal were statistically significant.

RESULTS

Descriptive Statistics

Body temperature of torpid animals was significantly lower than that of warm-active animals. Body temperature during torpor was not significantly different between groups. Body temperature on arousal from torpor and before euthanasia did not differ between groups and was not significantly different from that of warm-active animals. The rate of increase in the body temperature during arousal from torpor was also similar in all the arousal groups. Animals used in the arousal experiment all demonstrated normal active behavior after rapid arousal from torpor. No significant difference was found between the body masses of animals in the experimental groups (Table 1).

Effect of Acute Arousal on Lipid Composition of Dunnett Surfactant

Absolute lipid amounts. After 8 h of torpor, the absolute amounts of total Chol, PL, and DSP all increased (all measured as mg lipids/g DL) (Fig. 1, A-C). The changes were identical to those we documented earlier (13).

Table 1. Descriptive statistics of the experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>T(_{b}), °C</th>
<th>Rate of Arousal, °C/min</th>
<th>Body Mass, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8hT</td>
<td>31.3 ± 0.4</td>
<td>12.7 ± 0.3</td>
<td>11.9 ± 0.2</td>
</tr>
<tr>
<td>0 min</td>
<td>30.1 ± 0.4</td>
<td>15.3 ± 0.8</td>
<td>12.8 ± 0.6</td>
</tr>
<tr>
<td>5 min</td>
<td>30.0 ± 0.5</td>
<td>13.9 ± 0.6</td>
<td>12.7 ± 0.5</td>
</tr>
<tr>
<td>30 min</td>
<td>31.2 ± 0.6</td>
<td>14.5 ± 0.7</td>
<td>12.2 ± 0.2</td>
</tr>
<tr>
<td>60 min</td>
<td>31.7 ± 0.7</td>
<td>14.0 ± 0.5</td>
<td>11.8 ± 0.6</td>
</tr>
<tr>
<td>Data are means ± SE; n = 7–9 animals. T(_{b}), body temperature; WA, warm-active; 8hT, 8-h torpor. *Significantly different from WA group, P &lt; 0.05.</td>
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ately (0 min), 5, and 30 min after arousal (warm-active vs. 0 min, \( P < 0.002 \); 5 min, \( P < 0.001 \); and 30 min, \( P < 0.01 \)) but lower than in the torpid group (torpid vs. 0 min, \( P < 0.03 \); 5 min, \( P < 0.04 \); and 30 min, \( P < 0.04 \)) and reached warm-active values by 60 min after arousal (Fig. 1A). The rapid arousal from torpor did not alter the elevated absolute amounts of either total PL or DSP in surfactant. These parameters remained significantly higher than in the warm-active group at all time points studied and did not differ from those in torpid dunnarts (total PL, warm-active vs. 0 min, \( P < 0.02 \); 5 min, \( P < 0.001 \); and 30 min, \( P < 0.005 \); DSP, warm-active vs. 0 min, \( P < 0.01 \); 5 min, \( P < 0.001 \); 30 min, \( P < 0.006 \); and 60 min, \( P < 0.003 \)). The linear regression shows no change in these parameters over 60 min after arousal (Fig. 1, B and C).

Relative lipid amounts. After 8 h of torpor, Chol/DSP, Chol/PL, and DSP/PL ratios all increased (Fig. 2, A–C). The changes were identical to those we documented earlier (13). The Chol/DSP ratio returned to warm-active values immediately after arousal from torpor and decreased further over the 60-min period (Fig. 2A). Chol as a fraction of total PL immediately after arousal was higher than in the warm-active group (\( P < 0.03 \)). After 5 min of arousal, this parameter reached levels of warm-active animals and continued to decrease over the 60-min period (Fig. 2B). DSP as a percentage of total PL remained significantly higher than that in the warm-active group after rapid arousal from torpor (\( P < 0.05 \) at all time points) and did not change throughout the 60 min after arousal (Fig. 2C).

Effect of Acute Arousal from Torpor on the Surface Activity of Dunnart Surfactant

Adsorption rate at 37°C. Surfactant from torpid animals adsorbed more slowly than surfactant from warm-active animals at this temperature, which coin-
cides with our previous findings (15). Immediately after the rapid arousal from torpor, the adsorption rate did not differ from that of torpid animals and increased 60 min after arousal; however, it did not reach warm-active levels in this time period (Fig. 3A).

Adsorption rate at 15°C. The adsorption rate of pulmonary surfactant at 15°C was slower than at 37°C in all the groups studied (Fig. 3A and B). At 15°C, surfactant from torpid animals adsorbed faster than surfactant from warm-active animals. The adsorption rate changed immediately after arousal from torpor. In both 0- and 60-min groups, the adsorption rate was lower than in the torpid group but higher than in the warm-active group (Fig. 3B).

ST$_{min}$ and film compressibility at 37°C. Both ST$_{min}$ and film compressibility were higher in the 8-h-torpor group than in the warm-active group, which confirmed our previous findings (15). Immediately after arousal, ST$_{min}$ remained as high as in the torpid group; however, it rapidly decreased during the next 5 min after arousal (Fig. 4A). Film compressibility decreased immediately after acute arousal from torpor (0 min) and further decreased over 60 min after arousal; however, it did not reach warm-active values (Fig. 4B). The nature of changes in ST$_{min}$ and compressibility that occurred over the 60-min period after arousal from torpor is characterized by logarithmic and linear regressions, respectively (Fig. 4A, B). Representative surface tension-area isotherms (4th compression-expansion cycle) at 37°C for surfactant from warm-active, 8-h-torpor, 0 min after arousal, and 60 min after arousal groups (means of 2–3 animals) also demonstrate the changes in ST$_{min}$ and compressibility that occurred during torpor and after arousal (Fig. 6).
ST$_{\text{min}}$ and film compressibility at 15°C. Surfactant from warm-active animals had a higher ST$_{\text{min}}$ and compressibility when compared with that of surfactant from torpid animals. Both these parameters remained similar to those in torpid dunnarts for 60 min after arousal from torpor (Fig. 5, A and B).

**DISCUSSION**

Rate of Arousal From Torpor

In this study animals aroused from torpor within 15 min, during which time body temperature increased at an average rate of 1°C/min (Table 1). This rate is similar to the maximum rate of rewarming from torpor in this (S. crassicaudata) or similar species (8). All the animals survived rewarming and were active and alert over the postarousal period.

The body temperature of warm-active animals in the present experiment is slightly lower than the body temperature of those in a previous paper from our laboratory (15), which may depend on the time of year, body weight, and so on. Body temperatures of noneutherian mammals are characteristically labile. The heterothermic animals are considered active if they are able to maintain a body temperature of >30°C and demonstrate active behavior (running in the cage, looking for food, and so forth). Animals with a body temperature of <20°C are considered torpid (7, 16) and can usually be identified by a characteristic body posture (either lying

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**Fig. 3.** Effect of torpor and arousal on adsorption rates of dunnart pulmonary surfactant at different temperatures. A: 37°C. B: 15°C. Data are means of 6–7 animals (samples combined to give 2–3 measurements).
flat with splayed legs or curled tightly in a ball). In both our experiments, the fat-tailed dunnarts with a body temperature of 30–34°C were similarly active (and could bite equally well). We therefore consider the warm-active animals in the previous (13, 15) and in the present manuscript as a homogeneous group.

Surfactant Amounts and Composition

In our present and previous studies we used the Mason method to extract DSP (see Ref. 13). This method usually overestimates the DPPC content because it detects not only DSP but also some monoenoic species (29). However, the method is acceptable when used to compare values between experimental groups. In the present study we determined the total amount of DSP as opposed to DPPC. The percentage of DPPC in pulmonary surfactant of fat-tailed dunnarts can be calculated from the data in our previous study (13) and was recently estimated to be 56.9% of total PC (29), which is similar to that obtained in mammals by other researchers using the method of Mason (see Ref. 1).

The significant increase in the amounts of surfactant lipids during torpor in this study matched that demonstrated previously (13). During rapid arousal from torpor, the absolute amounts of Chol (in mg/g DL) decreased and immediately after arousal were significantly lower than in the torpid group, reaching warm-active levels by 60 min after arousal (Fig. 1A). In contrast, the absolute amounts of total PL or DSP did not change after arousal from torpor and remained at

Fig. 4. Effect of torpor and arousal on surface tension-lowering properties of dunnart pulmonary surfactant at 37°C. A: surface tension minimum (mN/m). B: film compressibility at a surface tension of 17 mN/m on compression (mN/m). Data are calculated from 4th compression-expansion cycle and expressed as mean of 6–7 animals. Time and animal euthanasia points after arousal are defined as in Fig. 1.
levels similar to those in torpid dunnarts (Fig. 1, B and C).

The ratio of Chol/DSP decreased rapidly during arousal and at 0 min was not significantly different from warm-active values (Fig. 2A). The Chol/PL ratio returned to warm-active levels by 5 min after the rapid arousal from torpor (Fig. 2B), whereas the DSP/PL ratio remained unchanged after 60 min after arousal (Fig. 2C).

It appears that the short time involved in arousing these animals from torpor (15 min) was associated with a rapid decrease in amounts of surfactant Chol, which, in turn, resulted in a significant decrease in both Chol/PL and Chol/DSP ratios to warm-active levels. These changes in surfactant Chol took only 15–20 min to occur, whereas changes in total PL and DSP require >75 min to become observable.

Surfactant turnover times vary from 3 to 10 h in adult mammals (rabbits, rats, and mice), in which ~50% of PL is reutilized (1). An increase in total PL in rat surfactant that occurred after swimming exercise takes at least 3 h to return to normal (23). Therefore, clearance of the elevated total PL and DSP levels after torpor possibly involves changes in PL synthesis, secretion, and reuptake and probably requires >75 min to complete, which is consistent with the slow changes in PL and DSP found in dunnart surfactant after arousal from torpor.

The rapid fall in Chol content (over the 20-min period) is fascinating. Although the mechanisms of such rapid Chol clearance are unknown, there is other evidence that surfactant lipid composition can change very rapidly. In lizards, the increase in absolute amounts of Chol and therefore in the Chol/PL ratio occurred

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**Fig. 5.** Effect of torpor and arousal on surface tension-lowering properties of dunnart pulmonary surfactant at 15°C. Figure description, definitions, and data calculation are the same as for Fig. 4.
within 2 h of a single step decrease in body temperature from 37 to 19°C (4). A number of studies in rats and humans suggest that Chol and PL are differentially regulated and that turnover times of these two components depend on the pattern of breathing and may change with activity of the animal (5, 19, 23). Hypopnea associated with exercise in rats caused a decrease in the Chol/DSP ratio, and these changes are evident after as little as 30 min (19). In humans the Chol/DSP ratio changes rapidly in response to 30-min exercise in a manner correlated with fitness. The Chol/DSP ratio decreases in the most-fit subjects, whereas in the less-fit subjects it increases (5). Our present findings provide more evidence that Chol and PL are handled differently in surfactant.

The source of Chol, as well as the mechanisms of its secretion into alveoli and reuptake, remains to be clarified. More studies are necessary to determine the mechanisms involved in the gradual increase in Chol content in surfactant during torpor and its rapid clearance in response to arousal from torpor.

Surface Activity

All the surface activity properties of dunnart pulmonary surfactant changed markedly both during and after acute arousal from torpor and corresponded with the rapid and slow changes in surfactant lipid composition. The surfactant from warm-active animals adsorbed faster than that in any other group at 37°C, whereas surfactant from torpid animals demonstrated a higher adsorption rate at 15°C (Fig. 3, A and B). Surfactant from animals killed immediately after arousal from torpor adsorbed slowly at 37°C, at a rate similar to that of surfactant from torpid animals, whereas by 60 min after arousal the adsorption rate at 37°C increased (but did not reach warm-active values). When measured at 15°C, adsorption rates of surfactant from animals aroused from torpor were intermediate (higher than that of the warm-active group but lower than that of the torpid group) (Fig. 3, A and B).

It is surprising that surfactant from torpid and 0-min animals with a high Chol content and those killed after arousal adsorbed more slowly at 37°C than did surfactant from warm-active animals. It is likely that some other changes occur in surfactant that affect adsorption, for example, alterations in the levels of surfactant proteins.

Surface tension-lowering capacity of surfactant from warm-active animals was higher at 37 than at 15°C. In contrast, after 8 h of torpor surfactant demonstrated a poorer surface tension-lowering ability at 37°C when compared with either the same surfactant measured at 15°C or with surfactant from warm-active dunnarts at 37°C, which is in agreement with our previous findings (15). There was a pronounced improvement in surface tension-lowering ability of surfactant at 37°C immediately and 5 min after acute arousal from torpor (a rapid decrease in STmin and compressibility); however, neither STmin nor compressibility reached warm-active values over the 60-min period (Figs. 4-6).

In this study an increase in the amount of Chol in surfactant found after 8 h of torpor was accompanied by increased rates of adsorption and improved surface tension-lowering ability of surfactant measured at 15°C. At 37°C there was a deterioration of these surface activity properties. The return of relative Chol amounts to warm-active levels by 5 min after arousal coincided with immediate improvement in surfactant surface activity at 37°C.

The functions of the neutral lipid classes, and Chol in particular, in pulmonary surfactant have not been clarified. Our findings support the suggestion that Chol facilitates surfactant adsorption and, together with increased DSP levels, improves surface tension-lowering ability of surfactant at 15°C. Chol enhances adsorption of DPPC, possibly by increasing fluidity, and improves film respreading at room temperature (6, 17). The removal of Chol from bovine surfactant hamper’s adsorption at 25°C (32). Simple mixtures of lipids containing Chol do not attain very low surface tensions on compression in the WLB (10, 11). Our previous study using the WLB at 20°C (15), and the present study in which we used the CBS at 15°C, both demonstrated that surfactant from torpid animals, highly enriched in Chol, possesses better surface tension-lowering ability at low temperatures than does surfactant from warm-active animals with lower Chol amounts. However, it is also possible that the concomitant increase in DSP/PL ratio found in these studies improves the surface tension-lowering ability of surfactant at 15°C.

Davidson et al. (3) observed that an in vivo increase in surfactant Chol/DSP ratio in rats, caused by the administration of 4-aminopyrazolo[3,4-d]pyrimidine, resulted in decreased surface activity (increased STmin and maximum surface tension) when measured in a pulsating bubble surfactometer at 37°C. We also observed a decrease in surface activity when we studied the surfactant from torpid animals at 37°C. We suggest that this poor surface activity relates to the elevated amounts of Chol in surfactant (i.e., increase in Chol/DSP ratio). At the same time, the addition of small amounts of Chol does not reduce the surface tension-lowering ability of bovine lipoid extract surfactant at 37°C in the CBS (21).

It is possible that the elevated amounts of Chol can be effectively squeezed out of the active surfactant monolayer, resulting in low STmin at 15 but not at 37°C. Chol shows preferential interactions with DPPC (12) and surfactant protein A (31). Because the pulmonary surfactant used in our study was not purified and probably contained all the surfactant proteins, it is possible that the interactions among Chol, DPPC, and proteins are temperature sensitive and more effective at 15 than at 37°C. Alternatively, the changes in the relative proportions of the surfactant lipids and possibly proteins, which occur during and after arousal from torpor, are responsible for the changes in the surface activity of surfactant and its temperature sensitivity.

Although the return of Chol to warm-active levels by 5 min after arousal corresponded with an immediate improvement in surfactant surface activity at 37°C, the
surface activity did not reach warm-active levels by 60 min after arousal. The elevated amounts of DSP that were maintained over this period were associated with a slow adsorption and increased ST\text{min} and compressibility of surfactant at 37°C, observed 5, 30, and 60 min after arousal. It is possible that the decreased amounts of USP, together with a decrease in the Chol/DSP ratio, resulted in the reduction in the amount of fluidizers in the surfactant, which impaired its ability to adsorb and respread at 37°C and therefore reduced its surface tension-lowering ability.

A surface tension of 13 mN/m at 37°C was found for surfactant obtained from torpid animals, killed after being in torpor for 8 h, with a body temperature of 15°C. The same surfactant at 15°C (i.e., equal to the body temperature of the animal) showed a surface tension of 1.8 mN/m. This data demonstrate that "torpid" surfactant works better at 15 than at 37°C and that we could assume that at 37°C such an animal would have died of respiratory distress syndrome. Another interesting question, however, is why the 0-min group, which consisted of animals with a body temperature of 30°C that appeared healthy and active, also had a surfactant with a low surface activity (i.e., ST\text{min} of 11 mN/m) at 37°C. It is possible that changes in surfactant lipid composition (and, very likely, in its protein composition) that occur during torpor and arousal result in the formation of a surfactant film with a very specific structure, which cannot be spontaneously reconstructed in vitro (i.e., when reconstituted lavage material is studied in the CBS). Alternatively, the high ST\text{min} that we found in the CBS in the 0-min group at 37°C may reflect the in vivo situation and may be accompanied by substantial compensatory changes in alveolar geometry and micromechanics during arousal in response to changes in metabolic rate, breathing frequency, and tidal volume.

The surface tension properties of pulmonary surfactant are essential for optimizing alveolar architecture to maintain sufficient gas exchange. Remarkably, even small changes in surface tension are associated with major changes in alveolar structure at the same volume.

Fig. 6. Representative surface tension-area isotherms for dunnart surfactant studied at 37°C. A: surfactant from WA and 8hT dunnarts. B: surfactant from dunnarts killed 0 and 60 min after arousal from torpor. Each curve represents 4th compression-expansion cycle. Data are expressed as mean of 2 or 3 animals.
(26, 27). Therefore, the changes in surface activity of surfactant found in this study and probably in the surface tension of the alveolar lining fluid would result in complex alterations in alveolar micromechanics and structure-function relationships in the lung in vivo. Because the respiratory parameters such as tidal volume and breathing frequency change during torpor and arousal (20) in dunnarts, it is possible that changes in lipid composition may be related to alterations in alveolar geometry that may occur during torpor and arousal in these animals.

In conclusion, the lipid composition and surface activity of pulmonary surfactant undergo biphasic changes in response to the rapid arousal from torpor in S. crassicaudata. There was a rapid change in the absolute amount of Chol, which resulted in a decrease in the Chol/DSP and Chol/PL ratios to warm-active levels immediately and 5 min after arousal, respectively. These changes coincided with a rapid improvement in surface activity. Then, slow changes in the amounts of total PL and DSP over the 60-min period after arousal followed, which were associated with more protracted changes in surface activity. However, neither lipid composition nor surface activity reached warm-active levels by 60 min after acute arousal from torpor. The clearance of the elevated PL and DSP levels after torpor presumably involve changes in PL synthesis, secretion, and reuptake and therefore require >75 min to complete. Our findings suggest that the elevated Chol can be cleared from the alveolar spaces within as little as 20 min and provide more evidence that Chol and PL in surfactant are differentially regulated. The mechanisms of this rapid clearance remain to be elucidated. Because these animals increase their body temperature at a rate of 1°C/min during arousal from torpor, the initial rapid changes in surfactant composition and surface activity may be necessary to optimize the alveolar surface area for gas exchange when rapid changes in pulmonary mechanics occur (e.g., an increase in tidal volume and breathing frequency). The slow changes in pulmonary surfactant toward warm-active levels may reflect a postarousal stabilization of the respiratory system at a higher body temperature.

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