we calculated an average daily metabolic rate (ADMR) at sea of 6.1 W kg^{-1} (Table 1).

The metabolic rate for a 13-kg penguin tending a chick is 4.3 W kg^{-1}; this rate is based on a mean weight loss of 0.15 kg day^{-1} (*9*). The ADMR at sea was about 1.5 to 2 times the ADMR of birds on the rookery. Since the SMR of a resting nonpasserine bird is 2.2 W kg^{-1} (*10*), our value of ADMR at sea is in good agreement with a value of two to three times the SMR determined for foraging terrestrial birds and mammals (*11*).

In comparing effort and success among the birds, we see that P5 expended half as much energy per time as P6, but it stayed at sea over twice as long. Overall, its total expenditure was average (Table 1). The dive data (Fig. 1) also show similar disparities of effort in that P1 made 1217 dives in 4 days (304 dives per day), and P3 made 890 dives in 8 days (111 dives per day). It would seem that at times birds go to sea for less time but work harder.

The three tritiated birds expended from 19×10^3 to 26×10^3 kJ of energy while at sea. This corresponds to the consumption of a total of 7 to 9 kg of squid (*12*). In February, an 8-kg chick is fed about every 4 days and it gets about 3 kg of squid per visit (*9*). Thus the energy cost of foraging is over twice as great as the energy content of the food delivered to the chick. Measurements of their beaks suggest that the squid taken probably weigh about 150 to 200 g. Therefore, during one trip, some 50 to 90 squid need to be caught to sustain the adult and feed the chick. With an average of 865 dives per bird per trip, a king penguin is likely to make a catch on fewer than 10 percent of the dives.

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4. V. H. Shoemaker, K. A. Nagy, W. R. Costa, *Physiol. Zool.* **49**, 364 (1976). The equation for calculating feeding rate from water turnover is

$$r_F = \frac{r_w}{P_w + E_F E_m M_w}$$

where r_F is the number of grams of dry food (F) consumed per kilogram of body weight per day, r_w is the total water turnover in milliliters per kilogram per day, P_w is the amount of preformed water per gram of F (= 4.0 ml per gram of F), E_F is the number of kilojoules of energy per gram of F (= 17.6 kJ per gram of F), E_m is the number of kilojoules metabolized per kilojoule of F ingested, and M_w is the water produced per kilojoule of F metabolized. The feeding rate (r_F) is converted to energy metabolized (in watts per kilogram) by

$$\text{ADMR} = r_F E_F E_m k$$

where k is a constant that converts the units to watts per kilogram ($k = 1.157 \times 10^{-8}$ W $day^{-1}kJ^{-1}$). The food is assumed to be squid, the composition of which was obtained from B. R. Watt and A. L. Merrill [*Composition of Food* (Agriculture Handbook No. 8, Department of Agriculture, Washington, D.C., 1963)]. The value of M_w (= 0.12 ml of water per kilocalorie of F metabolized) was calculated from the water produced from the oxidation of fat, carbohydrate, and protein [K. Schmidt-Nielsen, *Animal Physiology* (Cambridge Univ. Press, Cambridge, 1979)]. The value of E_m (= 0.80 kcal metabolized per kilocalorie of F ingested) was based on energy assimilation measurements of Adélie penguin chicks (*Pygoscelis adeliae*) as determined by D. P. Costa (unpublished data) and for other marine birds by E. H. Dunn [*Condor* **77**, 431 (1975)] and J. A. Kushlan [*ibid.* **79**, 31 (1977)].
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13. This work was supported by National Science Foundation grant DPP 78-22999 and National Institutes of Health postdoctoral fellowship PHS AM06093-01 to D.P.C. Logistic support was provided by the staff of the British Antarctic Survey to whom we are most grateful, particularly R. D. Bell and D. W. Doidge, who provided assistance in the field. D. M. Boone and W. Farwell gave considerable effort to final trouble-shooting and making ready the DHR's. We appreciate K. A. Nagy's review of an early draft of the report.

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Hypotensive Effect of Fasting: Possible Involvement of the Sympathetic Nervous System and Endogenous Opiates

Abstract. *Fasting lowers blood pressure to a greater extent in spontaneously hypertensive rats than in normotensive rats. While fasting reduced cardiac sympathetic activity to an equivalent extent in both groups of animals, only in the hypertensive rats did fasting elicit an opiate-mediated vasodepressor response that was independent of sympathetic withdrawal. Both sympathetic nervous system suppression and endogenous opiate activation, therefore, may contribute to the hypotensive effect of fasting in the spontaneously hypertensive rat.*

The hypotensive effect of fasting or caloric restriction is greater in hypertensive subjects than in normotensive subjects (*1–4*). In a recent study 4 days of fasting reduced systolic blood pressure 19 percent in spontaneously hypertensive (SH) rats and only 7 percent in the related normotensive Wistar-Kyoto (WKY) strain (*3*). Although numerous hypotheses have been suggested to account for this hypotensive response to fasting, none has gained general acceptance (*1–4*). The observation that fasting suppresses sympathetic nervous system (SNS) activity (*5*) has raised the possibility that the sympatholytic effect might contribute to the fall in blood pressure. We report here studies to assess the role of diminished SNS activity in fasting-induced blood pressure decreases in SH and WKY rats.

The effect of fasting on the turnover of tritiated norepinephrine in cardiac tissue (*6*) was measured simultaneously in SH and WKY rats after 4 days of fasting and during unrestricted feeding (Fig. 1). Although fractional and calculated turnover rates were significantly slowed by fasting in both strains, turnover rates did not differ in the two fed groups or in the two food-deprived groups. Fasting thus suppressed cardiac SNS activity to an equivalent extent in SH rats (−61 percent) and WKY rats (−65 percent), implying that mechanisms in addition to diminished SNS activity contributed to the greater hypotensive response of SH rats to fasting.

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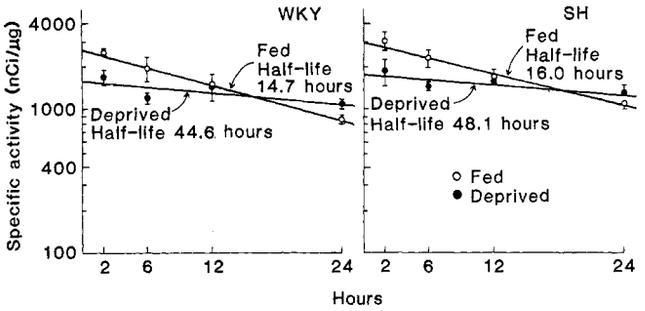

Fig. 1. Effect of fasting on turnover of tritiated norepinephrine in the hearts of SH and WKY rats. Norepinephrine turnover is a direct in vivo measure of SNS activity in sympathetically innervated organs of unanesthetized, unrestrained animals. Following its intravenous injection, the tracer is taken up into sympathetic nerves, rapidly equilibrated with intraneuronal norepinephrine stores, and released in response to incoming nerve impulses. The rate of disappearance of the tracer thus reflects SNS activity in an individual tissue. Ten-week-old male SH and WKY rats (Taconic Farms) were given unlimited standard rat feed (Charles River) and water or were given no feed and 50 m*M* NaCl to drink. Fasting began 4 days before the start of the turnover measurement and continued until the end of the experiment. On the day of study each animal received an intravenous injection of tritiated norepinephrine (200 μCi/kg; New England Nuclear). At preselected times four to five rats from each group were killed and their hearts were removed for analysis of tritiated and endogenous norepinephrine (*6*). Data are means ± standard errors for specific activity of norepinephrine in heart tissue in each group at each time. The line representing the decline in specific activity with time was calculated by the method of least squares. The slopes of the lines (fractional turnover rates) are 4.70 ± 0.68 and 1.55 ± 0.80 percent per hour for fed and food-deprived WKY rats, respectively ($P < .01$), and 4.33 ± 0.70 and 1.41 ± 0.76 percent per hour for fed and food-deprived SH rats, respectively ($P < .025$). The content of endogenous norepinephrine was 509 ± 26 and 593 ± 33 ng per heart in fed and deprived WKY rats and 634 ± 24 and 678 ± 34 ng per heart in fed and deprived SH rats. Calculated cardiac norepinephrine turnover (*6*) was 27.7 ± 5.5 and 9.8 ± 5.6 ng/hour in fed and deprived WKY rats (a difference of 65 percent) and 24.1 ± 4.7 and 9.5 ± 5.3 ng/hour in fed and deprived SH rats (a difference of 61 percent). Statistical evaluation was by analysis of covariance and Newman-Keuls multiple sample comparison (*15*). Fractional and calculated turnover rates did not differ significantly in the two fed or the two deprived groups.

A potential role for endogenous opiates in the hypotensive effect of fasting has been suggested in several reports. Inferential evidence of increased opiate activity during fasting has been obtained in studies of laboratory animals (*7*). In both animals and humans the administration of synthetic opiates or β-endorphin is associated with reductions in blood pressure and SNS activity (*8*). Moreover, endogenous opiates appear to participate in the antihypertensive response of SH rats, but not WKY rats, to centrally acting sympatholytic agents (*9*). These observations suggest that endogenous opiates might not only contribute to the lowering of blood pressure during fasting but might also account for the greater effect observed in SH rats.

To address the question of opiate involvement in the hypotensive response to fasting, we measured systolic blood pressure in 12-week-old male SH rats during unrestricted feeding and then, on the fifth day of fasting, before and 5 hours after subcutaneous injection of naltrexone (2 mg/kg), a long-acting opiate antagonist (Fig. 2A). The experiment followed a crossover design, with 2 weeks of unrestricted feeding separating the two trials. Since analysis of variance of the changes in systolic blood pressure in the two trials demonstrated no significant effect of crossover compared to replicate determinations, the data from the two trials were pooled. Systolic blood pressure fell from 187.5 ± 4.7 mmHg (feeding) to 161.1 ± 4.9 (fasting). Naltrexone increased systolic blood pressure 13.4 ± 3.8 mmHg over the ensuing 5 hours ($P < .02$); in contrast, no significant change was observed after saline treatment. Thus naltrexone increased systolic blood pressure in fasting SH rats, restoring it to levels approximately midway between the basal and fasting values. These data provide indirect evidence that endogenous opiates contribute to the reduction in systolic blood pressure observed in these animals during fasting.

To assess whether the pressor response to naltrexone was specific to fasting SH rats, we measured systolic blood pressure following administration of naltrexone or saline in SH and WKY rats during unrestricted feeding and after a 4-day fast and compared the results (Fig. 2B). Systolic blood pressure in naltrexone-treated, food-deprived SH rats increased significantly compared to that in the other three SH groups ($P < .005$). In WKY rats, on the other hand, no significant variation was noted among the four groups. The pressor effect of naltrexone was thus specific to food-deprived SH

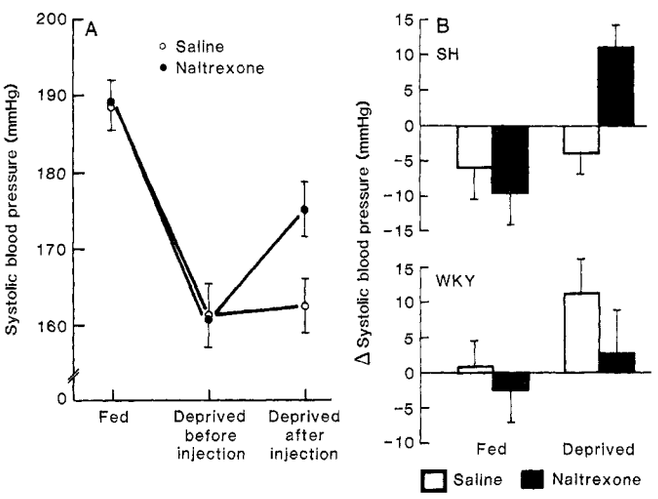

Fig. 2. Effect of naltrexone on systolic blood pressure in food-deprived SH rats. The animals were gently heated with a warming plate and heat lamp to a rectal temperature of 39°C, a procedure to which the animals had been acclimated over a 2-week period preceding the experiment. Systolic blood pressure in each animal was taken as the mean of eight measurements over a 5-minute interval. Blood pressure was measured by the tail-cuff method (*16*). (A) Systolic blood pressure in ten 12-week-old male SH rats during feeding and after 4 days of fasting. After the latter measurement, each animal was injected subcutaneously with naltrexone (2 mg/kg; Endo Laboratories) or an equal volume of saline. Five hours later blood pressure was again measured. After a 2-week interval of feeding the fasting protocol was repeated and the animals were given the opposite treatments. Data are means ± standard errors for the two SH groups. Naltrexone increased systolic blood pressure significantly (+13.4 ± 3.8 mmHg) ($P < .02$) in both trials, whereas saline did not. (B) Changes in systolic blood pressure in 16 SH rats and 16 WKY rats before and 5 hours after injection of naltrexone (2 mg/kg) or saline during feeding or after 4 days of fasting. All the rats were then fed for 2 weeks and the experiment was repeated in accordance with a partial crossover design. Half the rats in each group were given a diet or drug treatment different from that received in the first experiment. For example, of the rats given food and saline in the first experiment, half were given food and naltrexone and half were deprived of food and given saline. The data are means ± standard errors for the change in blood pressure over the 5 hours following drug administration in eight rats per group. In SH rats the variation in change in blood pressure among groups was significant [$F(3, 28) = 6.28$, $P < .005$], and the elevation in blood pressure after naltrexone was injected into deprived SH rats (+11.0 ± 3.1 mmHg) was significantly different from the responses in the other groups of SH rats ($P < .005$). In WKY rats the between-group variation in systolic blood pressure was not statistically significant.

rats. These data imply that the opiate-induced hypotension occurred only in the hypertensive animals and only in response to fasting. While the possibility that endogenous opiates also contribute to the fasting-induced reductions in systolic blood pressure in WKY rats cannot be dismissed, such an effect was not evident in WKY rats under the same conditions in which it was observed in SH rats.

If the actions of endogenous opiates in fasting SH rats (Fig. 2) include the reduction in SNS activity (Fig. 1), then the pressor effect of naltrexone in these animals should be associated with activation of the SNS. To examine this possibility, we measured the turnover of norepinephrine in SH rat hearts after 4 days of food deprivation. Half the rats received naltrexone after the administration of tritiated norepinephrine and the other half received saline. The rates of disappearance of the tracer from heart tissue did not differ between the two groups; the calculated norepinephrine turnover rates were 13.1 ± 4.1 ng per hour for naltrexone-treated animals and 18.5 ± 5.7 ng per hour for saline-treated animals (95 percent confidence intervals). Thus SNS activity, as measured by cardiac norepinephrine turnover, was not increased by naltrexone in unfed SH rats. While the pressor effect of naltrexone could be associated with SNS stimulation in a noncardiac tissue, preliminary experiments suggest that the blood pressure increase in food-deprived SH rats given naltrexone occurs even in the presence of alpha-adrenergic blockade (*10*). Thus the endogenous opiate mechanism activated by fasting in the SH rat does not appear to mediate the SNS suppression.

Previous attempts to explain the hypotensive effect of fasting focused on weight loss per se or a limitation in dietary sodium intake. Recent findings have tended to diminish the importance of these factors in the lowering of blood pressure induced by fasting in human subjects and SH rats (*1–4*), and the present results support two other mechanisms. The 4-day fast decreased SNS activity in the heart and did so to the same extent in both hypertensive and normotensive rats. Although these studies provide no evidence of a causal relation between SNS withdrawal and blood pressure reduction during fasting, such a connection is a reasonable presumption because of the intimate involvement of the SNS in blood pressure regulation. Fasting also elicited an opiate-mediated vasodepressor response, but one observed exclusively in the hypertensive animals. Since opiate antagonism in food-deprived SH rats only partially restored blood pressure to the levels measured before fasting, the additive effects of SNS suppression and opiate stimulation appear to have accounted for the greater hypotensive response to fasting in SH rats compared to WKY rats. Although similar changes in blood pressure and in biochemical indices of SNS activity appear to occur in dieting humans (*2*), no information is available to indicate whether opiate mechanisms are also operative.

Evidence suggests that the combination of SNS suppression with opiate activation is not unique to fasting SH rats (*7, 8*). Diminished SNS tone and increased opiate activity have been demonstrated in animals given 2-deoxyglucose, a nonmetabolizable glucose analog (*11*), and in genetically obese rats (Zucker *fa/fa*) and mice (*ob/ob*) (*12*). In traumatic injury, SNS activity decreases rapidly (*13*), a situation analogous to forms of experimentally induced hypotension (hemorrhagic or endotoxin shock) in which a role for endogenous opiates in blood pressure regulation has been inferred (*14*). Moreover, the frequently antagonistic effects of opiates and the SNS on blood pressure and metabolic rate suggest that reciprocal changes in the activity of both systems may be important in homeostatic regulation.

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Events in the Evolution of Pre-Proinsulin

Abstract. *An extensive computer-assisted analysis of known pre-proinsulin coding sequences has shown correlations that can be interpreted as evidence for an intron-mediated juxtaposition of exons in the evolution of these genes. The evidence includes the discovery that the regions of the pre-proinsulin genes that code for the signal peptide consist of nearly tandem repeating units of nine base pairs. This pattern reappears in the C region of the genes after a large intron that occurs in three of the four genes analyzed. A model is proposed in which primordial insulin was coded for by two separate minigenes arising from a gene duplication, each with identical or nearly identical signal peptide coding regions. The minigenes fused into one transcriptional unit mediated by the large intron, and the signal peptide coding region of one of the putative minigenes evolved into the latter portion of the C peptide coding region.*

The genomic structure of a number of pre-proinsulin genes have been elucidated. These include the human (*1*), the two rat variants—rat 1 and rat 2 (*2*)—and the chicken (*3*). These genes all contain a short intron in the 5′ noncoding region, and all except the rat 1 gene contain a long intron interrupting the region coding for the connecting peptide.

Gilbert (*4*) postulated that one function of introns is to bring together various exons to form new structural genes. The

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