Physiological inhibition and facilitation of adrenocortical response to hemorrhage

DONALD S. GANN, GEORGE L. CRYER, AND J. CARL PIRKLE, JR.
Departments of Biomedical Engineering and Surgery, and Division of Emergency Medicine,
The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

GANN, DONALD S., GEORGE L. CRYER, AND J. CARL PIRKLE, JR. Physiological inhibition and facilitation of adrenocortical response to hemorrhage. Am. J. Physiol. 232(1): R5-R9, 1977 or Am. J. Physiol.: Regulatory Integrative Comp. Physiol. 1(1): R5-R9, 1977. In a search for physiological feedback inhibition, secretion rates of cortisol were measured in intact dogs before and after sequential hemorrhages. The second of two sequential hemorrhages of 10 ml/kg separated by 90 min evoked significantly less increase of secretion rate of cortisol than did the first. This result was not explained by differential hemodynamic effects. Exhaustion of pituitary or adrenal capacities was excluded, since dogs responded normally to a second, larger hemorrhage. However, no attenuation of response to a second 10 ml/kg hemorrhage was seen after a larger, 20 ml/kg, first hemorrhage. This led in turn to a search for a physiological facilitatory mechanism which might offset the feedback effect. The second of two rapid sequential hemorrhages to isovolemia following preexpansion of plasma volume evoked significantly greater increase of secretion rate of cortisol than did the first. This result also was not explained by differential hemodynamic effects. The results support the hypothesis that hemorrhage elicits both physiological feedback and facilitatory effects which interact and which are (different) functions of the intensity of stimulus.

Adrenocorticotropic hormone (ACTH) has been postulated on the basis of inhibition of release of ACTH by administration of corticosteroids and of increase of release of ACTH following adrenalectomy, but the physiological importance of corticosteroid feedback in the immediate control of ACTH remains in doubt. In fact, in most experiments in which animals had been subjected to two sequential stimuli, no evidence for attenuation of the response to the second by the response to the first has been presented. Recently, Dallman and co-workers (4, 5) have postulated that a physiological feedback effect obtains but is offset by concurrent facilitation of the responsiveness of the control system brought about by the initial stimulus itself.

We have examined extensively the adrenocortical response to hemorrhage of varying magnitudes and rates and have shown that the release of ACTH elicited by hemorrhage and the suppression of release of ACTH elicited by infusion of exogenous steroids are not linearly proportional (10, 11). This lack of proportionality suggested that it might be possible to find some magnitude of initial stimulus at which a feedback effect might be unmasked, even if both feedback and facilitation were present in the control system. The present studies investigate this possibility.

METHODS

Experiments were conducted in 37 adult mongrel dogs weighing between 9 and 16 kg. On the day prior to the experiment and under pentobarbital anesthesia, the right lumboadrenal vein was cannulated by the method of Hume and Nelson (13). Femoral arterial and venous cannulas were placed at the same time. On the following day, under light pentobarbital anesthesia, the animal was allowed to stabilize for 2–4 h before experimental manipulations were begun between 9:30 and 11:30 A.M. Arterial pressure was measured by means of an arterial cannula, connected to a P23 DB Transducer and Brush Mark 200 eight-channel recorder. Blood was withdrawn through a second femoral arterial cannula at a constant rate by means of a Harvard peristaltic pump. Blood or other fluids were infused through a venous cannula at rates also controlled by pump. All hemorrhages proceeded at a constant rate as indicated below. All fluid infusions were also carried out at a constant rate over a 5-min period. Timed samples of adrenal venous blood were collected during control periods and 15 and 30 min after hemorrhage for determination of secretory rates of cortisol as 17-hydroxycorticosteroids (17-OHCS) by a modification of the method of Peterson, Karrer, and Guerra (16) or by radioimmunoassay (17). The sampling period ranged from 30 to 60 s. The two methods gave similar results for moderate to maximal secretion rates of cortisol. Secretory rates for cortisol were calculated as the product of adrenal venous concentration and adrenal blood flow. Adrenal blood flow did not change consistently or significantly after any of the hemorrhages used.

In all experiments accepted for analysis, control secretory rates of cortisol were less than 5 µg/min. Furthermore, adrenal responsiveness to ACTH was tested at the end of each experiment by infusion of a pulse of 100 mU of ACTH and collection of samples after 5 and 10 min. This dose of ACTH gives a maximum response. The mean response to ACTH (secretory rate after...
ACTH control) was $11.27 \pm 0.75$ (SE) $\mu$g/min. In all experiments accepted for analysis, secretory rates of cortisol after ACTH exceeded $8 \mu$g/min and the difference between control and post-ACTH secretory rates exceeded $5 \mu$g/min. Only 5 of 37 experiments were rejected by these criteria.

In the basic experimental design an initial hemorrhage was performed following collection of control samples. The rate of this hemorrhage varied and is described below. Subsequent adrenal venous samples were collected (15 and 30 min later); after sampling, blood was reinfused during a 5-min period. Samples were collected during the next hour, at which time a second hemorrhage was performed. Samples were collected at 15 and 30 min later as before and at this time ACTH was injected to test adrenal sensitivity. In some experiments, 1 h before the initial hemorrhage, 10 ml/kg of 6\% dextran in saline was infused. This degree of expansion of the plasma volume has been shown to be maintained for this period of time, as ascertained previously by sequential measurement of hematocrit. The hemorrhages that were performed subsequently were thus from a condition of hypervolemia to an isovolemic state. It has been shown previously that hemorrhage to isovolemia following this protocol of expansion of plasma volume is not an effective stimulus to secretion of cortisol if hemorrhage is slow (10 ml/kg per 3 min) but is a relatively small but consistent stimulus for fast (10 ml/kg per 45 s) hemorrhage (10). In most experiments, however, hemorrhage was either 10 or 20 ml/kg to a final state of hypovolemia. These stimuli have been shown to be consistently effective stimuli to secretion of cortisol of intermediate and maximum strength, respectively (11). Groups of data were compared through use of two-way analysis of variance corrected for repeated measurements on the same subject (18). Data are reported as means $\pm$ standard error.

**RESULTS**

**Sequential hemorrhages of 10 ml/kg to hypovolemia.** In eight dogs the hemorrhages were 10 ml/kg per 1.5 min. Both hemorrhages were of the same magnitude. This degree of hemorrhage is a consistent but submaximal stimulus to secretion of cortisol. The results of a typical experiment are shown in Fig. 1, which demonstrates that the second hemorrhage was considerably less effective than the first in eliciting secretion of cortisol. The mean responses of secretion of cortisol to the two hemorrhages are shown in Table 1. Analysis of variance indicated that the mean response to the second hemorrhage was significantly less ($P < 0.001$) than that to the first. The difference was not explained by a difference in control secretion rates. Arterial pressure fell $10.0 \pm 2.8$ Torr following the first hemorrhage and $10.3 \pm 2.6$ following the second. The range of change was $-22$ to $+2$ Torr after the first hemorrhage and $-23$ to $+4$ Torr after the second. Accordingly, at least by these criteria, the two hemorrhages appear equivalent hemodynamically. Thus, with two stimuli of the same magnitude separated by 90 min, the second stimulus appears considerably less effective than the first in eliciting release of cortisol.

![Graph showing cortisol secretion rates](http://ajpregu.physiology.org/)

**Hemorrhage of 10 ml/kg followed by 20 ml/kg.** The attenuation of the second response may have resulted from an inability of the pituitary-adrenocortical system to respond fully after the first response. To test this possibility a similar design was imposed in eight experiments in which the first hemorrhage was performed at 10 ml/kg per 3 min and the second hemorrhage was performed at 20 ml/kg per 3 min. The mean responses to the two hemorrhages are shown in Table 1. The response to the first hemorrhage was not significantly less than that to a first hemorrhage carried out in 1.5 min ($P > 0.1$). This confirms previous experiments which showed that rate sensitivity was not detected if final volume was reduced 10 ml/kg (8). There is no evidence of attenuation of the second response ($>0.75$). Thus, the pituitary adrenal axis appears to be responsive at this time after a first stimulus.

**Hemorrhage of 20 ml/kg followed by 10 ml/kg.** To test the possibility that suppression of the response to a second 10 ml/kg per 1.5 min hemorrhage resulted from physiological feedback, the magnitude of the first hemorrhage was increased to 20 ml/kg per 1.5 min. This stimulus has been shown to produce a maximum adrenocortical response, so that we expected to see a further decrease in the response to the second hemorrhage. The mean responses to the two hemorrhages are shown in Table 1. There was no evidence of attenuation of the second response. The mean response of six dogs to the second hemorrhage of 10 ml/kg following an initial 20 ml/kg hemorrhage was not significantly lower than that.
Sequential hemorrhages of 10 ml/kg to isovolemia. To test the possibility that physiological feedback was obscured in the previous experiments by an offsetting facilitatory effect of the initial stimulus, we examined the responses to sequential hemorrhages from hypervolemia to isovolemia at rapid rates (10 ml/kg per 45 s). Since the adrenocortical response to this stimulus is known to be small, we hoped to obtain a significant feedback signal by this choice of stimulus. As noted above, animals were preexpanded with dextran. The results of a typical experiment are shown in Fig. 2. The mean responses to the two hemorrhages are shown in Table 1. The response to the second hemorrhage was significantly greater than that to the first (P < 0.01). Arterial pressure fell 1.5 ± 3.0 Torr following the first hemorrhage, and 4.3 ± 2.5 Torr after the second. The range of changes was −17 to −15 Torr after the first hemorrhage and −17 to +12 Torr after the second. Accordingly, at least by these criteria, the two hemorrhages appear equivalent hemodynamically. Thus, with two stimuli of the same small magnitude separated by 90 min, the second stimulus appears considerably more effective than the first in eliciting increased secretion of cortisol.

**DISCUSSION**

The present studies demonstrate that there is an attenuated response to the second of two sequential 10 ml/kg hemorrhages separated by 90 min. This attenuation could have resulted from increased hemodynamic stability and thus resistance to the second hemorrhage. That this is not the case is suggested by the fact that changes in arterial pressure were equivalent in the two hemorrhages. Unfortunately, other cardiovascular variables which might reflect the effect of hemorrhage were not measured. However, to date, only changes in blood volume and in arterial pressure have been shown to influence release of ACTH (10, 11). Thus, from the point of view of release of ACTH, the idea of equivalence appears reasonable. Alternatively, the response to the second hemorrhage might have been attenuated as a result of limited capacity of the pituitary to release ACTII or of the adrenal cortex to respond to ACTH presented to it. That this is not the case is indicated by the larger response to a larger hemorrhage of 20 ml/kg at the same time after an initial 10 ml/kg hemorrhage. The results thus suggest strongly that the attenuation seen in the second 10 ml/kg hemorrhage results neither from a different stimulus nor from an incapacity of the system to respond. Accordingly, it seems most likely that physiological feedback accounts for the reduced response to the second stimulus.

If physiological feedback is the correct explanation for the attenuated second response, one might expect to see increased attenuation if the initial stimulus is larger and thus elicits greater response of secretion of cortisol. Instead, no attenuation of the response to a second hemorrhage of 10 ml/kg was noted if the initial hemorrhage was 20 ml/kg. This paradoxical result suggests that physiological facilitation may interact with the inhibition induced by cortisol to obscure the feedback effect, as postulated by Dallman et al. (4, 5). The result suggests further that such facilitation might be greater if the initial stimulus is greater.

If physiological facilitation is a characteristic of the system, one might expect to see it unmasked in a situation in which the initial stimulus does not elicit a large secretion of cortisol. Such a facilitation of a second response was found in experiments in which there were sequential rapid hemorrhages to isovolemia. However, since preexpansion with dextran was temporally closer to the first hemorrhage than to the second, and since dextran might have leaked out of the circulation or have been excreted, it is possible that the greater response was the result of a more severe hemodynamic insult. This does not appear to be the case, since changes in arterial pressure were equivalent in response to the two hemorrhages.

The criteria for the hypothesis of feedback control in the physiological sense are stringent and have rarely been met. To deduce both feedback control and its physiological importance, one would have to demonstrate that an initial physiological stimulus to the release of ACTH was followed by secretion of corticosteroid which in turn limited the subsequent release of ACTH in response to a stimulus. Recently, (2, 5) these criteria have been met for the prompt response to a stimulus in experiments in which ACTH was measured directly and was seen to fall rapidly following an initial response in intact but not in adrenalectomized animals. This evidence was taken to indicate the functional participation in the control system of the so-called fast feedback or rate sensitive element proposed previously by Dallman and Yates (8). However, a variety of attempts to demonstrate inhibition of a second stimulus by a first, at times which might be compatible with the operation of proportional or level sensitive delayed feedback have all failed (4, 5). Dallman and her co-workers showed that if corticosterone was infused in rats to levels which never exceeded physiological concentrations, then the release of ACTH following a later stimulus could be inhibited...
In contrast, a stimulus which led to even higher plasma concentrations of corticosterone with the same approximate time course did not inhibit the response to a subsequent stimulus. These results led Dallman et al. (4, 5) to suggest that the physiological feedback effects might be obscured by concomitant facilitation of the system, induced in some unknown way by the stimulus to release of ACTH itself. The present data are entirely consistent with this suggestion, but indicate that with appropriate choice of stimulus it is possible to unmask either the feedback or the facilitation effect.

A phenomenon which may be equivalent to facilitation has been described previously. Dallman and Yates (7) found that rats prestressed by a scald responded to a second stimulus with histamine in the presence of blockade by dexamethasone which would prevent the response if there were no initial stress. Ondo and Kitay (15) obtained similar results with dexamethasone-blocked rats in which urethan anesthesia served as the initial stress. Witorsch and Brodish (19) showed that previously stressed rats would respond to a new stress in the presence of median eminence lesions which blocked the response to an initial stress. The facilitation suggested the opening of a new pathway not included in the lesion. Daniels-Severs et al. (9) have demonstrated a potentiating effect of chronic stress lasting up to 8 wk and present in the absence of enhanced adrenal responsiveness to ACTH. Thus, it is possible that the facilitation of release of ACTH by an initial stimulus not only interacts quantitatively with feedback inhibition, but also leads to qualitative and persistent changes in the behavior of the control system. However, the present experiments shed no light on these conditions.

The unmasking of feedback or of facilitation depends on the relations among stimulus, concentration of cortisol, and response. In previous studies of the adrenocortical response to hemorrhage in intact animals, we have demonstrated that the steady-state secretion of cortisol 15–30 min after hemorrhage is proportional to the logarithm of the magnitude of hemorrhage (10, 11). Further studies, in which the adrenocortical response to hemorrhage of low magnitude was studied after prior infusion of dexamethasone at various rates, demonstrated that the degree of inhibition of release of ACTH in response to small hemorrhage was inversely proportional to the square root of the rate of steroid infusion (11). A similar mathematical relationship between naturally secreted steroid and suppression of release of ACTH has not been demonstrated. However, if the general form of the mathematical relationships obtains, there is no linear proportionality that can obtain over the entire scale of stimulus and response that relates a possible stimulus effect to a possible feedback effect. It is this lack of proportionality that permits the choice of stimulus to unmask feedback and facilitation in the present experiments.

In studies in the rat, Henkin and Knigge (12) noted decreased output of corticosterone 12 h after stimulation with continuous sound. On the basis of other experiments, these workers believed that adrenal exhaustion and depletion of pituitary ACTH could both be excluded. Feedback could not be excluded completely but did not seem likely because a further increase in response was noted if the stimulus continued. It is possible, however, that in this work there may have been a temporal interaction of feedback and facilitatory effects which allowed the observation of a feedback effect at a single point in time. The alternative explanation offered by Henkin and Knigge, that change in central neural activity obtained, is really equivalent to this sort of interaction. In other studies in the rat, Bohus (1) noted decreased production of corticosterone at various times after hemiadrenalectomy (12 h) or after immobilization (4 h). He showed that there was a further decline in production rates when corticosterone was injected exogenously. These results also suggest a complex interaction of a possible steroid feedback effect with the effect of a stimulus in potentiating secretion. Such studies do not demonstrate but do suggest that unmasking of feedback effects may be possible for stimuli other than hemorrhage, provided an appropriate choice of times for observation is made. However, recent evidence has again raised the possibility of diminished capacity of the pituitary to release ACTH some hours after a major stress (6), so that there is an alternative explanation to feedback for the these experiments.

Dallman and Yates (8) showed that there are distinct, fast (first 10 min) and delayed (after 2 h) inhibition of response to histamine after physiologic rates of infusion of corticosterone in the rat. They termed the rapid and transient inhibition "fast feedback." The existence of rate-sensitive feedback in the rat has been confirmed by Jones, Brush, and Neame (14), who showed that the period of inhibition extended beyond that initially described by Dallman and Yates, and who defined certain quantitative and temporal features of this effect. The present experiments offer no evidence on the existence or nonexistence of fast feedback in the dog. However, they do suggest strongly that the delayed, level-sensitive feedback effect is present in the dog and may be elicited as early as 90 min after an initial stimulus.

The present experiments thus suggest that facilitation and feedback by cortisol, both induced by an initial stimulus, play a physiological role in modulating the adrenocortical response to a subsequent stimulus. The quantitative relations among stimulus, facilitation, secretion of cortisol, and the feedback effect of cortisol determine ultimately the relative effectiveness of a second stimulus. Depending on the outcome of these interactions, one might conclude that a system exhibited feedback, facilitation, or neither; whereas it seems likely that both effects are important physiological features of adrenocortical control.

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