

# Behavioral effects of intracerebroventricular infusion of luteinizing hormone releasing hormone (LH-RH) in pigeons

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Pigeons were implanted with stainless steel cannulae in the lateral ventricles of the brain and infused with different concentrations of LH-RH and CF insulin, as well as with saline, in counterbalanced order. LH-RH elicited dose-dependent effects in the animals' behavior, and CF insulin resulted in much weaker reactions; the saline infusion generally had no effects. These results suggest that LH-RH could nonspecifically regulate a wide range of complex behavior.

Morphological and physiological data indicate that the ependyma, an epithelium that covers the internal surface of cerebral ventricles and separates the cerebrospinal fluid (CSF) from surrounding nervous tissue, may also have a secretory function (Kiss & Mitro, 1978; Leonhardt, 1969; Mitro & Kiss, 1977; Vigh, 1971). The CSF appears to serve as a vehicle for a number of hormone-like substances secreted by adjacent cerebral structures (Knowles, 1974). For instance, in mammals, nucleus striae terminalis neurons have been shown to contain luteinizing hormone releasing hormone (LH-RH)<sup>1</sup> (Silverman, 1977). LH-RH is also found at high concentrations in the organon vasculosus laminae terminalis (Okon & Koch, 1977), a periventricular organ, and is present at reasonable titers in the CSF itself (Morris, Tandy, Sundberg, & Knigge, 1975). In the pigeon, neurosecretory granules have been demonstrated in the nucleus striae terminalis by resorcin-fuchsin staining (Delius, 1975). Moreover, histological studies of this last structure in birds make probable its similarity with some of the classical circumventricular organs and suggest that the nucleus striae terminalis may secrete into the lateral ventricles.

In addition, it has become increasingly apparent that some hypothalamic hormonal peptides play physiological roles in the specific neuronal integration of complicated behaviors. In particular, although the behavioral significance of the LH-RH is largely unknown, the widespread occurrence of neurons sensitive to LH-RH has already led to the speculation that it might be involved in the control of more varied behaviors (Moss, 1977). Its role in the modulation of sexual

behavior has been reported in rats (Moss & McCann, 1973; Pfaff, 1973; Riskind & Moss, 1979), in ring doves (Cheng, 1977), and even in humans (Evans & Distiller, 1979). All those observations, taken in conjunction with our work stimulating or lesioning the nucleus striae terminalis in birds [its stimulation in gulls yielded threat responses followed by a persisting aggressive mood, and lesions of the same structure in pigeons largely abolished aggressive responses to external stimuli (Delius, 1973; Martin Ramirez & Delius, 1979b)], suggested that the agonistic changes observed after such manipulations might be related to the concentration of the presumptive neuropeptide released into the ventricular lumen. In other words, the possible secretion of LH-RH in the nucleus striae terminalis and its release into the ventricles might be somehow involved in the control of aggressive behavior.

As an initial test of this hypothesis, we undertook to observe the effect of intraventricular injections of synthetic LH-RH upon the behavior of pigeons. As a vehicle control, we infused physiological saline and another decapeptide with a quite different structure: CF insulin.

## METHOD

Four adult homing pigeons of unknown sex were used. They were kept in individual cages, at a nearly constant temperature of 18°C and on a 12-h light, 12-h dark cycle. Their daily food consumption was recorded. Cannulae made of hypodermic needle tubing were implanted into the lateral ventricles while the animals were anesthetized and their heads were held in a stereotaxic apparatus. When the cannulae were correctly located, CSF was expelled from the ventricles. Then a stylet plug was fitted to the inner diameter and length of the needle, and the cannula was cemented into the skull with dental acrylic.

After 1 week of recovery, trials were conducted in the home cages each midmorning, 5 days a week for 12 weeks. On an alternative schedule, we injected LH-RH, CF insulin solutions, or isotonic saline infusion. The formula of the saline was adjusted to be equivalent to avian CSF. The doses of both decapeptides were increased progressively over the trials from

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1 to 1,000 micrograms. For the trial, the stylet plug was removed from the cannula and a small-diameter tubing prefilled with the injecting solution was run through the cage roof and connected to the cannula. After 15 min, a .1-ml solution was injected over 60 sec with the aid of a Hamilton microsyringe equipped with a screw drive. After 30 min, the tubing was removed and the cannula was again closed with the stylet plug.

The anal temperature was measured at the beginning and at the end of each session. The experimenter kept the animal under continuous observation for 30 min, making detailed notes on its behavior. Agonistic behavior was measured by the pigeon's response to the introduction of a stick or the experimenter's hand into its cage, as described in detail elsewhere (Martin Ramirez & Delius, 1979a).

## RESULTS

Regarding aggressive and more generally agonistic behavior, the animals failed to provide substantive evidence for consistent changes, except that this group of behaviors was suppressed by the behavioral syndrome we describe below, as were feeding, drinking, preening, and courting. Food intake and body temperature remained at normal levels.

The graded intensities of the syndrome observed are illustrated in Figure 1, which plots the mean dose-response curve. For quantification purposes, the behavioral responses were rated according to a scale of four points: 0 = lack of response. 1 = weakest response; this consisted of a sequence or combination of eye blinking, gaping, head shaking, neck twisting, tail lowering, wing shivering, and so on, lasting only 1-2 min and starting some 5-10 sec after the intraventricular injection began. 2 = medium response; this also incorporated complete eye closure, loss of motion, loss of balance, and drowsy crouching for some 3-5 min. 3 = strongest response; this included jerking shivers, rigidity, and complete loss of balance. The pigeons fell down dramatically, striking the ground with their beaks and tumbling over onto their backs with a head-to-tail rotation. Balance was recovered within about 5 min,

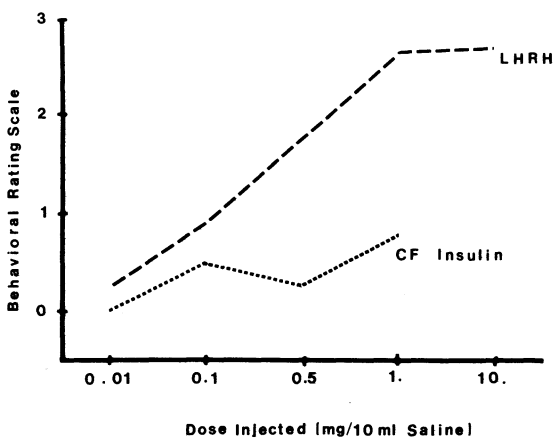


Figure 1. Mean score of the behavioral changes after each dose. Explanation of the rating scale is in the text.

but the animals remained in a crouched position for as long as 30-60 min. Their eyes remained closed or half closed for at least 15 min, and occasionally the animal scratched its nictitating membrane, even after several hours. Consequently, the nictitating membranes became irritated, and sometimes the surrounding area bled.

Although the results (Figure 1) suggested a dose-dependent effect, this was not absolute; not every subject responded with the same reaction to the same quantity of peptide infused. Comparative doses of both decapeptides elicited different degrees of responses: CF insulin at best yielded only a weak effect. The saline infusion generally had no behavioral effects.

## DISCUSSION

It now appears widely accepted that neuropeptides have important influences on the integrative functions of the CNS and, consequently, in the regulation of behavior (Pribram, 1977; Yarbrough, 1979). One example of this could be the general behavior syndrome described here, which is clearly abnormal, especially in the more intense form that follows higher dose infusion, and which is characterized by gross motor disturbances. The possibility that we might have been witnessing an anaphylactic response seems remote, since the onset was nearly immediate after injections and, furthermore, there was no corresponding rise in body temperature.

The dramatic effects on the motor system described as "strongest response" appear to have some similarity to the epileptoid reactions reported to follow the intraventricular infusion of other neuropeptides. More specifically, the immobility and muscle rigidity of our birds appear somewhat like the epileptic stupor of the rigid and akinetic rats reported after intraventricular injections of TRH, somatostatin, and beta-endorphin (Cohn & Cohn, 1975; Cohn, Cohn, & Taylor, 1975; Havlicek & Friesen, 1979; Havlicek, Rezek, & Friesen, 1976; Havlicek, Rezek, Leybin, Pinsky, & Friesen, 1977). Moreover, the violent close head-to-tail rotations induced appeared similar to the tonic-clonic seizures with opisthotonus and extension and other epileptogenic effects described by the same authors, especially those unusual rotations reported after TRH injection (Cohn & Cohn, 1975). Similar examples of possible interactions between different neurally active peptides have been stated by Yarbrough (1979).

The fact that several neuropeptides affect the same kinds of behavioral responses, although to different degrees and even sometimes with antagonistic patterns (for example, somatostatin antagonized TRH in the regulation of the body temperature, narcosis, and locomotor activity; see Cohn & Cohn, 1975; Cohn et al, 1975) suggests the hypothesis that nonspecific regulation of a wide range of complex behaviors is accomplished by maintaining a constant balance of polypeptide activity in a number of neural circuits.

Intraventricular administration of LH-RH did not have any notable, acute, specific effects on the agonistic behavior of pigeons. These data, however, do not necessarily mean that such a role for this neuropeptide could not exist, but only that more sophisticated techniques and further work in multi-disciplinary fields are required to understand its nature. For instance, our group is pursuing this research through a micro-structural and immunocytochemical study of the ventricular system, ependyma, and circumventricular organs. These preliminary results thus remain tentative until more data and control studies become available, and they do not exclude the possibility that LH-RH in the CSF might have more subtle effects on the behavior of pigeons.

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## NOTE

1. LH-RH is a decapeptide known also as luteinizing hormone releasing factor (LH-RF) and gonadotropin releasing hormone (GN-RH).

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