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Relationship Between Prostagandin E_1, Polyphloretin Phosphate and α and β Adrenoceptor-bound Feeding Loci in the Hypothalamus of Sheep

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BAILE, C. A. AND F. H. MARTIN. *Relationship between prostaglandin E_1, polyphloretin phosphate and α and β adrenoceptor-bound feeding loci in the hypothalamus of sheep.* PHARMAC. BIOCHEM. BEHAV. **1**(5) 539–545, 1973.–We have previously proposed that prostaglandins (PG) may play a modulating role on hypothalamic areas controlling feeding and energy balance. In the present experiment we have tested in the medial hypothalamus of sheep for interactions between α and β adrenoceptors, PGE_1 and a PG antagonist polyphloretin phosphate (PPP). Sheep were prepared with 6 cannula guides in the hypothalamus. In each sheep following preliminary injection with l-norepinephrine (l-NE) and dl-isoproterenol (dl-Isop), loci were selected that showed preferentially either α or β adrenoceptor-bound feeding. In the subsequent experiment PGE_1 blocked the l-NE (α-agonist) elicited feeding in the α-bound feeding loci but PGE_1 elicited feeding when injected into the β-bound feeding loci. The PGE_1-elicited feeding was specifically blocked by a β antagonist (LB-46). The PPP elicited feeding in both α and β-bound feeding loci but the responses were blocked only by the α antagonist (phentolamine) in the α loci and by the β antagonist (LB-46) in the β loci. These responses lend support to our previous conclusions that injection of α agonists into some and β agonists into other hypothalamic sites, but not vice versa will elicit feeding. PGE_1, injected into loci showing differences in sensitivity to adrenoceptor agonists which elicit feeding, results in increased feeding, as in this experiment, or decreased feeding as shown in a previous report. Thus, we conclude that although it is unlikely that systemically produced PG modulate hypothalamic controls on feeding and energy balance because of the dual effect on feeding, there may be an interaction of endogenous hypothalamic PG and adrenoceptors.

Feed intake l-norepinephrine dl-isoproterenol LB-46 Phentolamine

WE HAVE shown that feeding can be elicted in sheep by intraventricular or intrahypothalamic injections of either α or β adrenoceptor agonists [5]. The agonists tested included a universal adrenoceptor agonist, l-epinephrine (l-Epi) [2], relatively specific α agonists, l-norepinephrine (l-NE) and phenylephrine [5], general β agonist, dl-isoproterenol (dl-Isop) and l-isoproterenol [5], and relatively specific β_2 agonists, Salbutamol and 3, 4-dihydroxy-β-hydroxyphenethyl-tert-butylamine [1,2]. The αelicited feeding responses required approximately 50–100 times as much of the l-isomer as required for the β agonists via either of the test routes [5]. l-Epi had a biphasic dose response with the higher dose range clearly showing an α-elicited feeding while the lower dose range had some of the characteristics of the β-elicited feeding [1,5]. The feeding elicited by α adrenoceptor agonists was blocked by phenoxybenzamine or phentolamine, while the feeding elicited by β agonists was blocked by dl-propranolol or LB-46 [1,5].

In addition, we have shown that in some of the loci where l-NE elicited feeding in sheep, PGE_1 decreased intakes whereas polyphloretin phosphate (PPP), a possible PG antagonist [6,13] increased feeding [1]. PG reportedly block l-NE transmission [11] and the metabolic effects of l-Epi [12]. Also PG have been proposed as a part of the α adrenoceptor system [22].

In the present experiment we have selected, in each sheep, hypothalamic loci in which injections of (1) an α adrenoceptor agonist or (2) a β adrenoceptor agonist elicited feeding. These loci were tested with combinations of PG and α and β agonists and antagonists and PPP. Our objective was to use several combinations of these agents to further characterize the relatively specific loci for feeding. We were also interested in possible body temperature changes elicited by these drugs since adrenoceptor agonists and PG alone can affect the body temperature of some species.

METHOD

A total of eight sheep was tested in these experiments. Each sheep was surgically prepared under aseptic conditions with three guides on each side 1.5 mm lateral to midline; the guides were 1 mm apart in the rostrocaudal plane. The guides, made of 20 gauge tubing, were implanted 26 mm below the dura in rostrocaudal and lateral planes determined by X-ray and held in place with acrylic resin. Except during injection procedures a stylet was in place in each guide.

The sheep were held in elevated stalls with free access to water in a room maintained at 23±1 C and with a 40% humidity. They were fed ad lib a 60% concentrate pelleted ration [21]. The feed was removed briefly at intervals during the tests for weighing.

After recovery from surgery and resumption of normal 24-hr feed intake, 240 nmoles of l-NE in 1 μl of carrier was injected into each sheep unilaterally at sites approximately 4 mm from the base of the brain. The six sites were tested until one was found which resulted in at least 100 g intake in the first 30 min. Such a site was tested with the carrier solution (C), 0.9% NaCl, on the following day. The testing procedure was repeated using 8 nmoles of dl-Isop to deter-

TABLE 1

THE TREATMENTS AND ORDER TESTED. EACH SHEEP WAS INJECTED IN α AND β BOUND FEEDING LOCI. EACH INJECTION WAS FOLLOWED BY AT LEAST 48 HR DURING WHICH NO INJECTIONS WERE GIVEN. THE INJECTIONS WERE ALTERNATED BETWEEN THE α AND β SITES.

Treatment				Treatment Order		n
1st Injection	Dose (n Moles)	2nd Injection	Dose (nmoles)	Group I	Group II	
			α BOUND FEEDING SITES			
C*	–	NE	240	11	13	7
C	–	C	–	25	25	7
C	–	Isop	8	14	1	7
PGE_1	21	C	–	9	9	8
PGE_1	21	NE	240	3	7	8
PGE_1	21	Isop	8	1	5	8
PPP	6	C	–	5	3	6
PPP	6	NE	240	7	11	7
PPP	6	Isop	8	12	8	6
Phent	6	PPP	6	17	17	6
LB-46	6	PPP	6	19	19	7
Phent	6	C	–	21	21	7
			β BOUND FEEDING SITES			
C	–	Isop	8	16	16	7
C	–	C	–	26	26	7
C	–	NE	240	13	15	7
PGE_1	21	C	–	4	6	8
PGE_1	21	Isop	8	8	2	7
PGE_1	21	NE	240	15	12	8
Phent	21	PGE_1	21	22	22	6
LB-46	21	PGE_1	21	20	20	6
LB-46	21	C	–	18	18	7
PPP	6	C	–	6	14	7
PPP	6	Isop	8	2	4	6
PPP	6	NE	8	10	12	7
Phent	6	PPP	6	24	24	6
LB-46	6	PPP	6	23	23	6
Phent	21	C	–	27	27	6

*C = 0.9% NaCl

mine β-bound feeding loci. The doses of l-NE and dl-Isop selected were based on previous experiments [5].

At the completion of the screening for α and β responsive sites, the sheep were randomly divided into two groups. The series of tests shown in Table 1 were in the order listed for the two groups. All tests were followed by at least a 48-hr no-test period and the sheep were injected at α and β loci on alternate test days. All injections were of 1 μl and the various doses and drugs tested are given in Table 1. With a few exceptions, each locus tested in each sheep was injected with each drug. In those tests involving 2 injections the first preceded the second by 5 min. This interval was selected based on our work and that with rats [14].

One hour prior to each test the sheep were given fresh feed which in nearly all tests stimulated a spontaneous meal by each sheep. This meal helped to synchronize the feeding pattern at the time of injection.

All sheep were also surgically prepared with tubes (8 mm ODx20 cm length, Silastic Medical-Grade tubing, Dow Corning Corp) inserted into the upper left abdominal fossa. The proximal end was sealed with cement (Medical Adhesive, Silicone Type A, Dow Corning Corp) and a dacron mesh skirt was cemented about 5 cm from the open distal end; the mesh was sutured under the skin. Prior to tests, a thermistor was inserted into the intraperitoneal tube of each sheep and the deep body temperatures were scanned with a YSI Telethermometer.

At the end of the experiments the sheep were sacrificed with succinyl choline. The head of each was perfused with saline followed by 10% formalin via the carotid arteries. The injection loci were marked with 1 μl of indelible ink and the brains were removed from the skull. The hypothalami were examined grossly and then prepared for histology.

Feeding and temperature data were tested for significant differences by analyses of variance (Animal x Treatment) and Multiple Range Tests.

RESULTS

Figure 1 shows the feed intakes following injections of α and β adrenoceptor agonists and PGE$_1$. The 1 μl carrier (0.9% NaCl) injections were followed by feed intakes not different from those during a no-injection test. l-NE (240 nmoles) in the α-bound feeding loci caused much greater feed intakes than the control even in the first 15 min. The latencies of the feeding response in all of these tests were similar and were about 5 min. The feeding response of the sheep following injections in these same loci with dl-Isop (8 nmoles) was less at all time intervals ($p<0.05$). In these

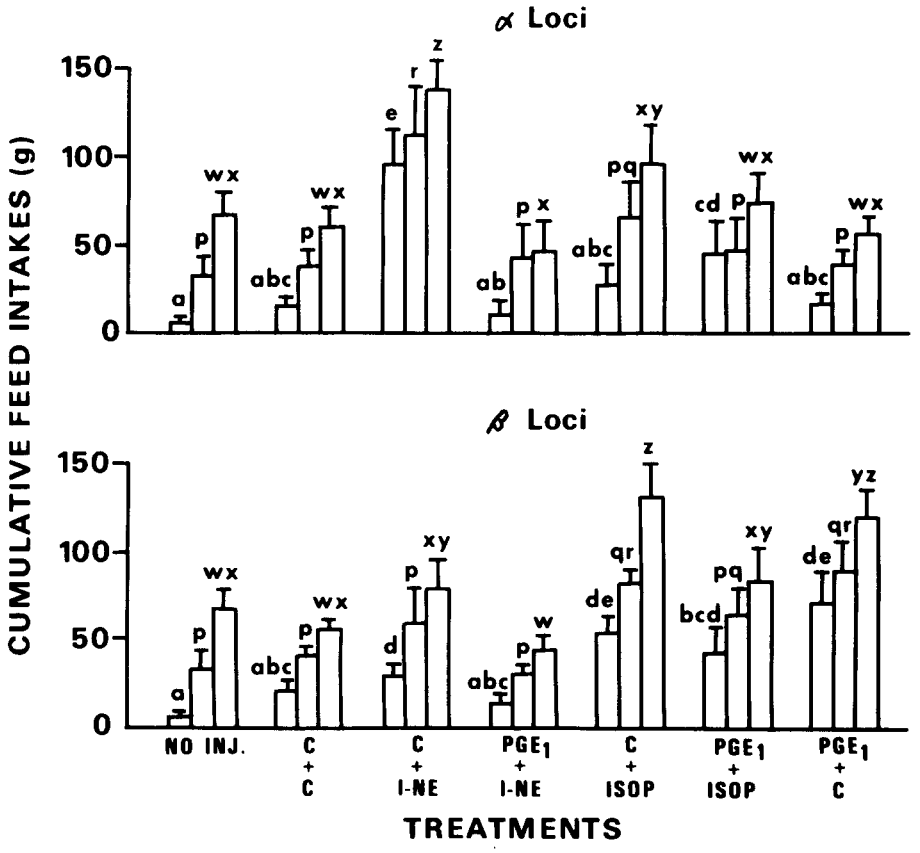

FIG. 1. Feed intake responses following injections into sheep in hypothalamic loci preselected for α- and β-bound feeding. The groups of 3 bars show 15, 30, and 60 min cumulative intakes. The 2 injections (1 μl) were given 5 min apart. Means within a time period which do not share a common letter are different ($p<0.05$; n for each treatment given in Table 1). Treatment abbreviations: C = control; l-NE = l-norepinephrine (240 nmoles); PGE$_1$ = Prostaglandin E$_1$, (21 nmoles); Isop = dl-Isoproterenol (8 nmoles).

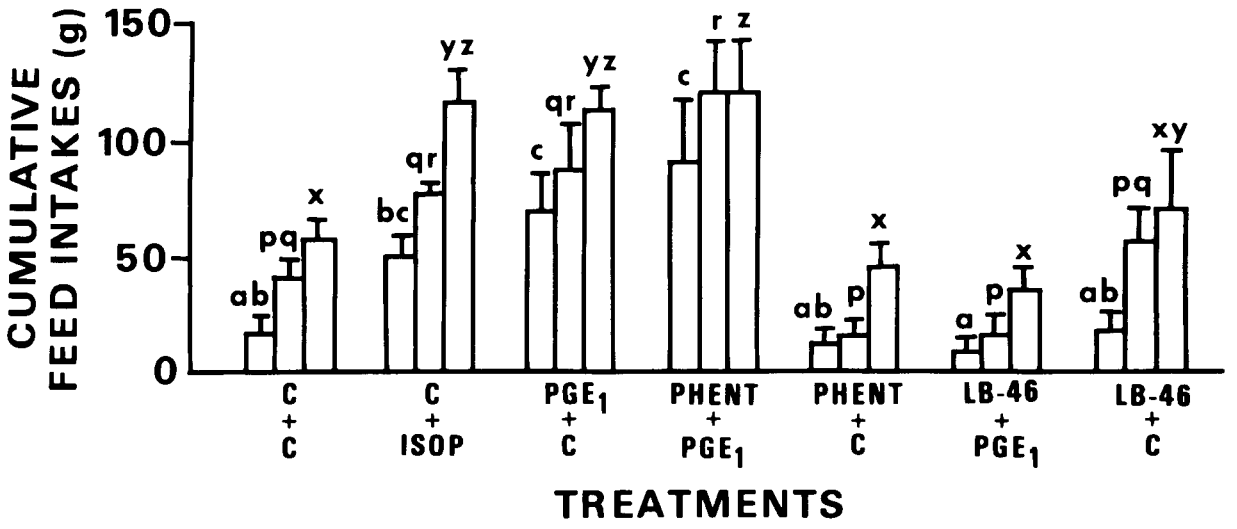

FIG. 2. Feed intake responses following injections into hypothalamic loci showing β-bound feeding. The groups of 3 bars show 15, 30 and 60 min cumulative intakes. Means within a time period which do not share a common letter are different ($p<0.05$; n for each treatment given in Table 1). Treatment abbreviations: C = control; Isop = dl-Isoproterenol (8 nmoles); Phent = Phentolamine (21 nmoles); LB-46 (21 nmoles).

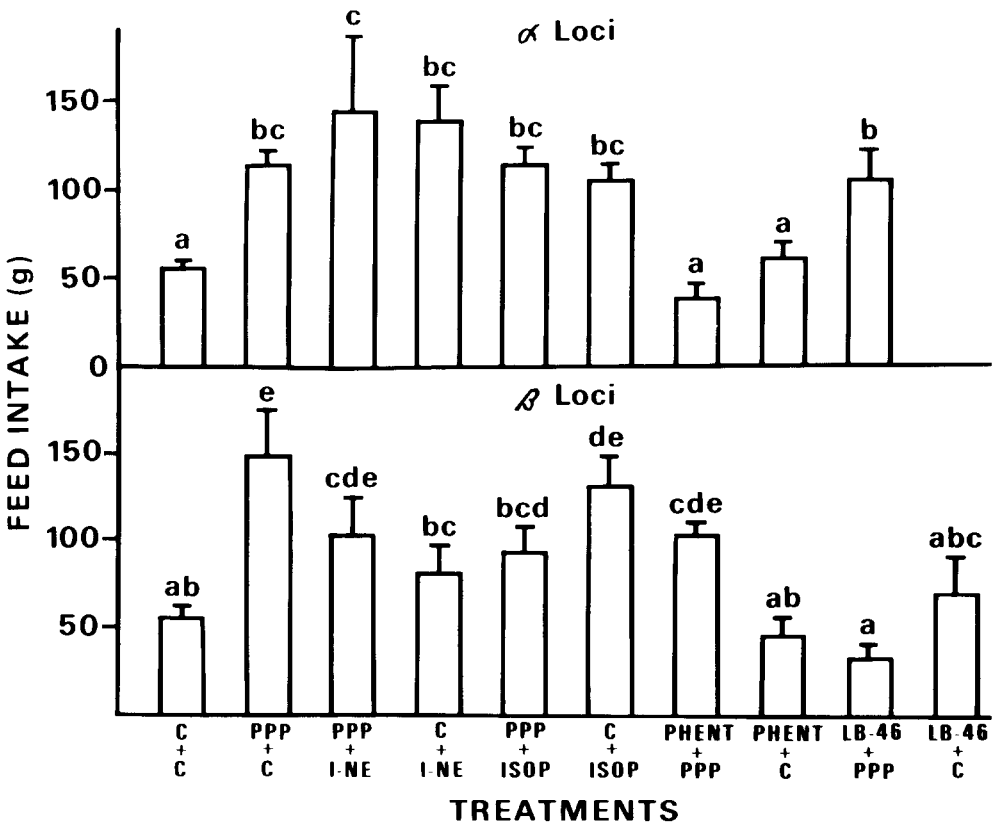

FIG. 3. The 60-min feed intake responses following injections into hypothalamic loci of sheep showing α- and β-bound feeding. The 2 injections were givern 5 min apart. Means which do not share a common letter are different ($p<0.05$; n for each treatment given in Table 1). Treatment abbreviations: C = control; PPP = polyphloretin phosphate (6 nmoles); l-NE = l-norepinephrine (240 nmoles); Isop = dl-Isoproterenol (8 nmoles); Phent = Phentolamine (6 nmoles); LB-46 (6 nmoles + PPP and 21 nmoles + C).

same sheep, injections of dl-Isop into loci showing predominantly β-bound feeding elicited greater intakes than l-NE injections at the 30 and 60 min time intervals, ($p<0.05$). The l-NE feeding response in the α-bound feeding loci was nearly equal to that following dl-Isop in the β-bound feeding loci. The response to l-NE injection in the β-bound feeding loci was less than the following l-NE in the α-bound feeding loci at each of the 3 time intervals ($p<0.01$). l-NE in the β-bound feeding loci was followed by intakes similar to those measured following dl-Isop in the α-bound feeding loci.

PGE_1 injected 5 min prior to l-NE in either α or β-bound feeding loci resulted in only normal feeding which was less than feeding in response to control injections followed by l-NE ($p<0.05$). PGE_1 injected prior to dl-Isop into the α-bound feeding loci did not significantly change the feeding from that of dl-Isop alone. However, the same combination in the β-bound feeding loci resulted in less feeding than that observed with dl-Isop alone in these loci ($p<0.05$). Although PGE_1 + C injected into the α-bound feeding loci did not affect the normal feeding, the same injection into the β-bound feeding loci elicited feeding greater than control or that observed in the α loci ($p<0.05$).

Figure 2 shows additional tests on the PGE_1 elicited feeding. Injections in all these tests were made only into the β-bound feeding loci. Injection of 21 nmoles of phentolamine prior to PGE_1 did not reduce the feeding response. Injections of LB-46 (21 nmoles) prior to PGE_1 reduced the feeding response to that of the control level. Neither phentolamine + C nor LB-46 + C injections changed significantly the control feeding.

Figure 3 shows the effects of polyphloretin phosphate and α and β adrenoceptor agonists and antagonists on feeding. PPP injections elicited feeding in both α- and β-bound feeding loci but a greater response in the latter. The feeding response obtained with the combination of l-NE and PPP injections into the α loci was as great as that following l-NE alone. The same combination into the β loci resulted in an intermediate response between PPP + C and l-NE + C. The feeding responses of PPP in combination with dl-ISOP were similar to those of PPP and l-NE. Phentolamine (6 nmoles) injected into the α-bound feeding loci followed by PPP reduced the PPP feeding response to a control level while the same combination in the β-bound feeding loci resulted in feeding ($p<0.05$). However, LB-46 (6 nmoles) injected prior to PPP into the β-bound feeding loci blocked the PPP elicited feeding. This was not the case in the α-bound feeding loci since LB-46 did not reduce the PPP elicited feeding.

Although internal temperature was measured in each experiment, no significant changes from the control or the no-injection treatments were observed.

Figure 4 displays the histological placements of each injection locus. We could not discern any anatomical group of α or β loci and must conclude that they were similarly dispersed throughout the hypothalamus.

DISCUSSION

In these experiments we have found support for the results of our previous experiments showing feeding elicited by injections of α and β adrenoceptor agonists into specific loci. In the present experiment, loci showing specificity for α and β agonist-bound feeding selectively responded to injections of PGE_1, i.e. only PGE_1 elicited feeding in the β-bound feeding loci. This response was blocked by the β antagonist and not by the α antagonist. In addition, although PPP, a possible PGE_1 antagonist [6,13], elicited feeding when injected in either α or β feeding loci, the feeding responses were selectively blocked by α and β antagonists, respectively. The injection of two drugs which by themselves elicited feeding did not have an additive effect in our tests presumably because both activated the same system.

The doses of PGE_1 tested were based on those found to be effective in our previous experiments [1]. The doses of antagonists required have been generally the same as the agonists tested in our previous experiments and therefore a similar ratio of either PGE_1 or PPP and α and β adrenoceptor antagonists was maintained. Several of our experiments have shown that the antagonists do not affect normal feeding [1].

Although PGE_1 appeared to act as an antagonist to l-NE in α-bound feeding loci, it is not clear that PGE_1 was acting on the apparent β adrenoceptor in our system in a way similar to that described by Hoffer, Siggins and Bloom [11] in the Purkinje cells. The PGE_1 response studied by Hoffer, *et al.* was apparently that of an antagonist of β adrenoceptors while the feeding response in our tests appears to be the results of PGE_1 playing a β agonist role. However, we cannot be certain that the apparent β adrenoceptors which elicit feeding in response to dl-Isop are the same ones involved in the PGE_1-elicited feeding. That LB-46 blocks both the dl-Isop and PGE_1-elicted feeding is taken as supporting evidence that the same type of receptors are involved. In a previous experiment, injection of PGE_1 into loci which showed both α- and β-bound feeding resulted in decreased feed intake [1]. Presumable the loci tested in the present experiment were sufficiently different from those previously tested that neither type exhibited the decreased feeding response to PGE_1 injections observed earlier We are not yet able to show that any one hypothalamic area has predominantly one type of adrenoceptors.

The specific inhibition of PPP by the α and β antagonists is additional evidence showing the selectivity of the α- and β-bound feeding loci. It was not feasible to test a variety of doses of each antagonist at these two types of adrenoceptor-bound feeding loci. At best, we can claim a difference in the blocking effect with the minimal dose of antagonists between these types of loci. That both PGE_1 and PPP elicited feeding in the β-bound feeding loci may suggest that PPP, at least at these loci, is not acting through the same mechanism and PPP may have other effects on neuronal function than that related to PGE_1 antagonism. It should be noted that the doses used in the present experiment must be considered much higher than normal production rates. Although physiological levels of PG may play a role in modulating the adrenoceptors involved in controlling feeding or energy balance, the role is probably not that of a plasma feedback which has been proposed in earlier papers [4,20]. It is interesting that blood PG concentrations may vary with feeding activity [10], i.e., increase during and following a meal and decrease during fasting. It is difficult to visualize a feedback system, which controls feeding or energy balance by involving systemic tissues, in which PGE_1 has direct action on β adrenoceptors because increased PG would result in increased feeding or at least in a lowering of the hunger threshold. However, endogenous PG in the hypothalamus may still play a modulating role on the neuronal balances controlling feeding.

The apparent α and β adrenoceptor feeding responses are possibly related to the various vasculature responses [23]

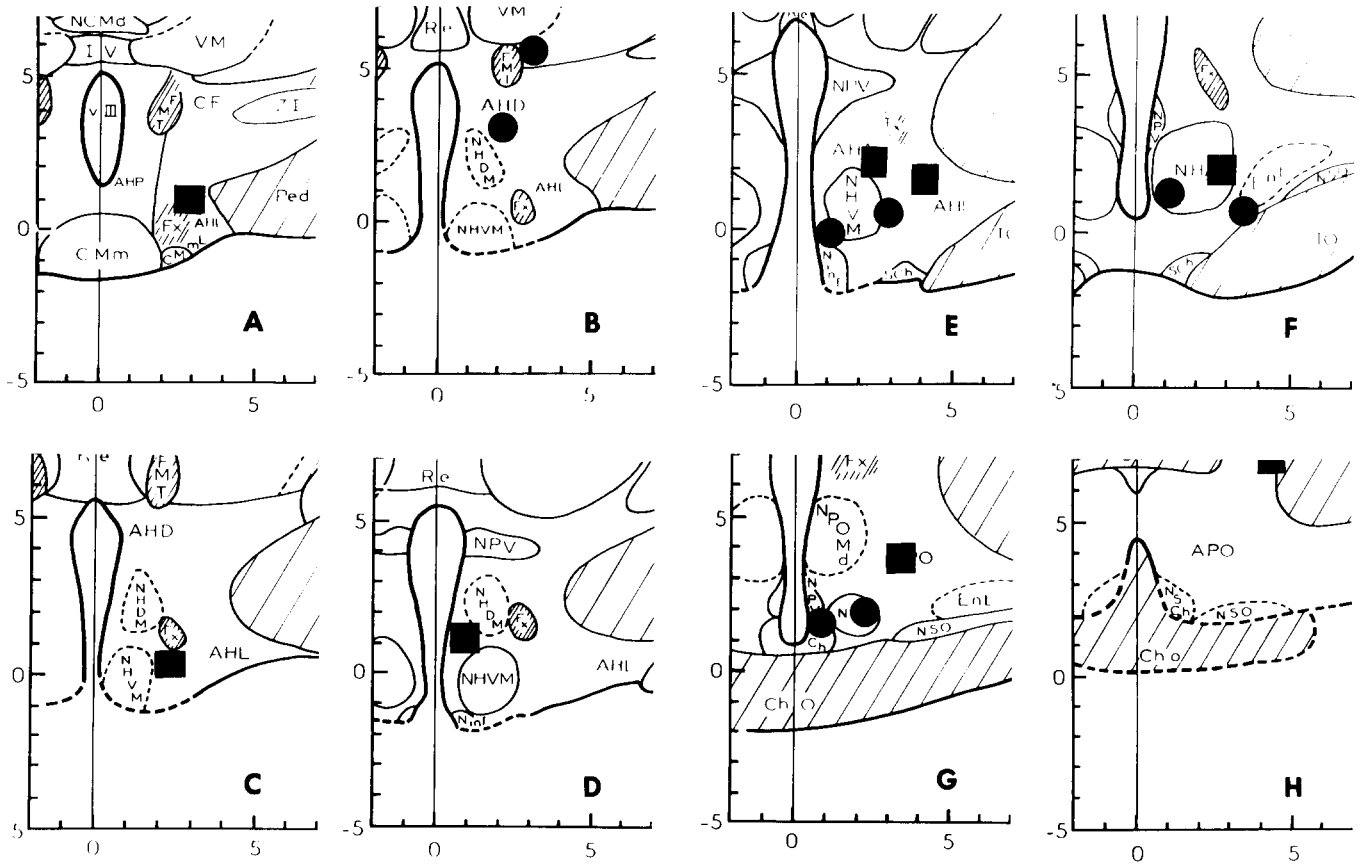

FIG. 4. Histological summary of injection loci. The circles and squares each show an α- or β-bound feeding locus, respectively. The schematics are from the atlas of the sheep brain by Richard [19]. A, B, C, D, E, F, G and H are 25, 26, 27, 28, 29, 30, 31 and 32 mm anterior to the plane of the internal auditory canal.

that the agents used in these experiments might cause in the CNS. It seems unlikely that this is an explanation for the relatively clear α and β loci differentiation.

The central body temperature regulatory centers of sheep are apparently unresponsive to the doses of drugs used in these experiments. Both l-NE and PGE_1, especially, have been shown to influence body temperatures of cats [8, 15, 18], rabbits [15], and rats [4, 16, 18] and monkeys [17].

We conclude from this series of experiments that both α and β adrenoceptors and possibly cholinergic [1,9] and 5-HT [7] receptors are involved in the neural chains controlling feeding in sheep. In animals with apparent α- and β-bound feeding loci as those tested in the experiments reported here, it would be interesting to know the effect of an injection of one agent in one type of locus and a second injection of another agent into the other type of locus to show dependence or independence of these two apparently different adrenoceptor systems. The nature, e.g., metabolic, taste, of the type of information of which any of these circuits may be adding to the integrated feeding drives is not known [1].

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The chemicals used in these experiments were obtained from the following sources: l-NE (-) Arterenol bitartrate hydrate B grade – Cal Bio Chem (M.W. = 337.3); dl-Isoproterenol HCl – Winthrop Laboratories (M.W. = 247.7); PGE_1 – Dr. John E. Pike, Upjohn Company (M.W. = 354); Polyphloretin Phosphate – SK&F (Approximate M.W. = 15,000); Phentolamine HCl – Ciba Pharmaceuticals (M.W. = 317.71); LB-46 (dl-4-(2-hyroxy-3-isoprotyl-aminopropoxy)-indol – Sandoz Pharmaceuticals (M.W. = 229).

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