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Central Effects of CCK Ligands in Pigs Making Operant Responses for Food

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PARROTT, R. F. *Central effects of CCK ligands in pigs making operant responses for food.* PHARMACOL BIOCHEM BEHAV **49**(3) 463–469, 1994.—The effects, on operant feeding, of intracerebroventricular (ICV) injections of the CCK_A receptor agonist A71623, the CCK_B receptor agonist A63387, and the CCK_A receptor antagonist A70104 were investigated in prepubertal pigs. In overnight starved animals, feeding was inhibited by 20, 5, and 1 μg doses of A71623 and by 20, but not 5 or 1 μg doses of A63387. In a second experiment, although pigs pretreated centrally with A70104 (20 μg) showed a tendency to eat more, the effect was not statistically significant. Furthermore, this dose of A70104 did not prevent the inhibition of feeding induced by a subsequent ICV injection of CCK (1 μg). These findings support the view that exogenous CCK reduces food intake in pigs by acting, primarily, on CCK_A receptors.

Pigs Cholecystokinin (CCK) receptors Central injection Operant feeding

PREVIOUS research in this laboratory has established that both intravenous (IV) and intracerebroventricular (ICV) administration of cholecystokinin (CCK) suppresses operant feeding in pigs (4,13). In an attempt to determine whether these effects are mediated by action at the CCK_A or CCK_B receptor, a recent study (12) investigated feeding behavior in pigs given IV or ICV injections of the A agonist A71378 or the B agonist pentagastrin. The results showed that whereas only A71378 reduced operant feeding when given peripherally, neither A71378 nor pentagastrin significantly affected food intake after ICV administration, although CCK itself produced a marked inhibition when given by this route. As a possible explanation for these results, it was suggested that these agents may have been inactivated by central peptidases (12). Studies using other receptor ligands that might be less readily metabolized, therefore, seemed to be indicated.

In addition to A71378 and pentagastrin, the tetrapeptides A71623 and A63387 have been shown to exhibit high selectivity for the CCK_A and CCK_B receptors, respectively (10,11). Moreover, inhibition of feeding after peripheral administration of the CCK_A agonist A71623 has been demonstrated in rodents and carnivores (1–3) but not in primates (9). This peptide also reduces food intake when given centrally to rats (3) and baboons (9). In contrast, the CCK_B agonist A63387 has negligible effects on feeding when given peripherally to rats (3) or centrally to baboons (9) and rats (3). In view of these findings, and the inconclusive effects of A73178 and pentagastrin when given ICV to pigs (12), it was thought to be of interest to examine the effects of ICV administration of A71623 and A63387 in the pig operant feeding model.

The role of CCK in the control of feeding in pigs has also been studied using the CCK_A antagonists devazepide and A70104, the dicylohexylammonium salt of a compound derived from proglumide (2). However, whereas IV devazepide stimulates operant feeding (6), A70104 given by the same route is ineffective in this respect (7). Also, although ICV devazepide has been shown to enhance food intake in pigs (5), there is little information available about the central effects of A70104 on feeding. Hence, a second objective of this study was to determine whether this antagonist would increase appetite in pigs.

METHOD

The animals used were 20 large white (16 male, 4 female) prepubertal pigs supplied at an initial weight of approximately 25 kg. They were individually housed in metabolism cages where they learned to make operant responses for food (weaner pellets) and water by pressing switch panels with their snouts. Each pig was prepared under general anesthesia, using sterile precautions, with an ICV (lateral ventricle) cannula. However, as there is no stereotaxic atlas available for the pig, cannulae placements were confirmed by the ability of ICV angiotensin II (250 ng in 0.5 ml saline) to induce marked operant drinking (mean reinforcements ± SEM; 44.7 ± 4.1, Experiment 1; 78.0 ± 18.9, Experiment 2). Food availability was signaled by a buzzer and food was obtainable on a fixed ratio of five presses for each reinforcement from 1000 to 1100 h and from 1600 to 1630 h, whereas water was continuously available. Testing took place during the normal morning feed-

ing session with the operant responses from each animal displayed on a chart recorder.

In Experiment 1, three groups of pigs were given ICV injections of vehicle and 20 μg ($n = 6$), 5 μg ($n = 4$), or 1 μg ($n = 7$) of the CCK_A agonist A71623 or the CCK_B agonist A63387, using a semirandomized design. The vehicle used was sterile water containing 10% dimethyl sulfoxide (DMSO), and all injections, which were of 0.3 ml vol, were followed by 0.5 ml sterile saline. In each case, injections were given 5 min after the start of feeding.

In Experiment 2, pigs ($n = 5$) were given ICV injections of the CCK_A antagonist A70104 (20 μg in DMSO vehicle), or vehicle, 15 min before the start of feeding. The animals then received a second injection of vehicle or CCK (1 μg) 5 min after feeding had commenced and behavioral responses were recorded for a further 40 min. Thus, the pigs received either vehicle plus vehicle, A70104 plus vehicle, A70104 plus CCK, or no treatment.

The number of food reinforcements (mean ± SEM) obtained in each 5-min period of the various test sessions, during the 25-min postinjection period in Experiment 1, and throughout the whole of the 45-min feeding period in Experiment 2, was calculated. The total amount of food eaten under the different treatment conditions of both experiments was compared using analysis of variance. Where significant treatment effects were found, differences between individual treatments were compared using Tukey's test, and the results are expressed as two-tailed probability values.

RESULTS

Figures 1–3 illustrate the findings of Experiment 1. In tests with the highest (20 μg) dose of the CCK receptor agonists (Fig. 1), the number of reinforcements obtained in the 5 min before the ICV injection was similar in each treatment condition. The rate at which subsequent reinforcements were delivered decreased gradually during the next 25 min when the pigs were given vehicle. However, this decrease was more marked after treatment with the CCK_B agonist and rapid following ICV injection of the CCK_A agonist. Analysis of variance detected significant ($p < 0.002$) differences between the treatments with respect to total intake in the postinjection period (Fig. 1). Subsequent comparisons indicated that total intake after vehicle was greater than that after either the CCK_A ($p < 0.01$) or CCK_B ($p < 0.05$) agonist. However, there was no difference between these two treatments with regard to the total amount of food consumed.

A similar pattern of feeding activity was evident when pigs received the intermediate (5 μg) dose of the CCK_A and CCK_B receptor ligands (Fig. 2). Again, there were significant ($p < 0.02$) treatment differences in overall intake during the postinjection period. However, the amount of food eaten after the CCK_A agonist was less than that consumed after the vehicle ($p < 0.05$) but did not differ from that recorded when the pigs were given the CCK_B agonist (Fig. 2). Total intakes after vehicle and CCK_B agonist treatments were also not significantly different.

The effects of the lowest (1 μg) doses of the peptides are shown in Fig. 3. Under these conditions, vehicle and CCK_B agonist treatments produced similar patterns of operant responding, whereas the CCK_A agonist once again induced a rapid cessation of feeding. Overall treatment effects were significant ($p < 0.01$), and the amount of food consumed after ICV injection of the CCK_A agonist differed ($p < 0.05$) from the totals recorded after administration of the vehicle or the CCK_B agonist (Fig. 3). However, there was no difference in the amount food eaten after vehicle or CCK_B agonist treatments.

The findings of Experiment 2 are indicated in Fig. 4. Feeding decreased gradually over the 45-min test period in pigs pretreated with the vehicle or in the absence of treatment. There was a tendency for A70104 to increase food intake slightly throughout the trial, but pretreatment with this dose of the drug did not antagonize the inhibitory effect of ICV CCK. Analysis of variance revealed a significant ($p < 0.001$) effect of treatments on total food intake during the whole of the 45-min test period. Subsequent comparisons indicated that there were no significant differences between the amount of food consumed in the absence of treatment and after ICV injection of vehicle or A70104 (Fig. 4). However, when CCK was given after pretreatment with A70104, total food intake was reduced compared with the occasions when the animals either received no treatment ($p < 0.01$) or were given vehicle ($p < 0.05$) or A70104 alone ($p < 0.01$).

DISCUSSION

In contrast to earlier observations in pigs treated with other CCK receptor ligands (12), the present results indicate that central administration of the CCK_A agonist A71623, or the CCK_B agonist A63387, reduces operant feeding.

Previous research has shown that consumption of liquid food in rats (3) is inhibited by ICV injection of A71623 (0.6, 1.25, or 5.0 nmol) or A63387 (5 or 10 nmol). Nonoperant food intake in baboons is also reduced by A71623 (11 nmol ICV), but not by a 10-fold larger dose of A63387 (9). Similarly, 1, 5, and 20 μg ICV doses of A71623 (equivalent to 1.7, 8.3, and 33.3 nmol, respectively) suppressed operant feeding in overnight fasted pigs, whereas only the highest dose of A63387 (20 μg; 33.3 nmol) reduced meal size. In a variety of species, therefore, A71623 seems to be at least 10 times more potent than A63387 with respect to a central inhibitory action on feeding activity.

There is evidence from studies in other species to indicate that the size of the peripheral dose of A71623 required to produce inhibition of feeding is greater than the effective ICV dose. For example, in baboons, 110 nmol decreases food intake after ICV, but not after IV, injection (9). Also, in experiments where A71623 has been given intraperitoneally (IP), the effective doses for the reduction of meal size in hungry animals are 3 nmol/kg (rats; 1,3), 10 nmol/kg (mice, monkeys; 2), and 30 nmol/kg (dogs; 2). On this basis, an IP dose of not less than 75 nmol would be required to suppress feeding in pigs of the size used in this study. Moreover, data from rats indicate that the IP route is about 30 times more effective in producing a reduction in meal size than the IV route (1). Such considerations, therefore, suggest that the findings reported here represent central effects of these CCK agonists because, even if there had been leakage into the peripheral circulation, the quantities would have been too small to have had any behavioral effect.

There was no clear dose–response relation for A71623 in Experiment 1 because each dose produced a cessation of feeding within 10 min. This suggests that, as in the rat (3), ICV injection of doses smaller than 1 μg may inhibit food intake in pigs. Such a finding would not be surprising given the potency of A71623 and the sensitivity of the pig operant feeding paradigm. Equimolar ICV doses of CCK and A71623 produce similar reductions in food consumption in baboons (9); therefore, as 1 μg CCK given ICV to pigs reduces operant feeding

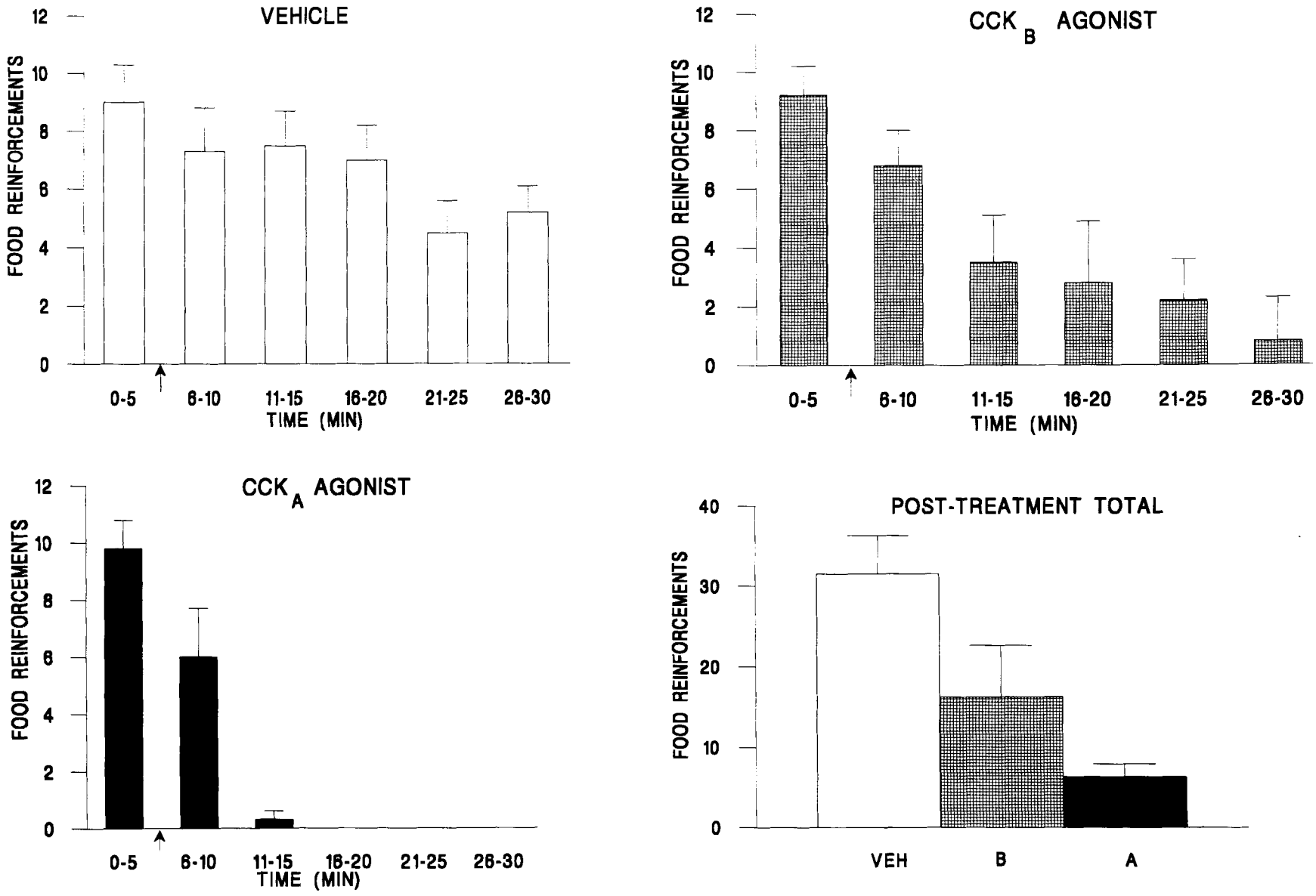

FIG. 1. Food reinforcements (mean ± SEM) obtained in 5-min periods before and after ICV injection (indicated by the arrow), and overall posttreatment totals, in pigs given vehicle or 20 μg CCK_A or CCK_B agonist in Experiment 1. Post-treatment totals were significantly different, $p < 0.002$ (see text for further details).

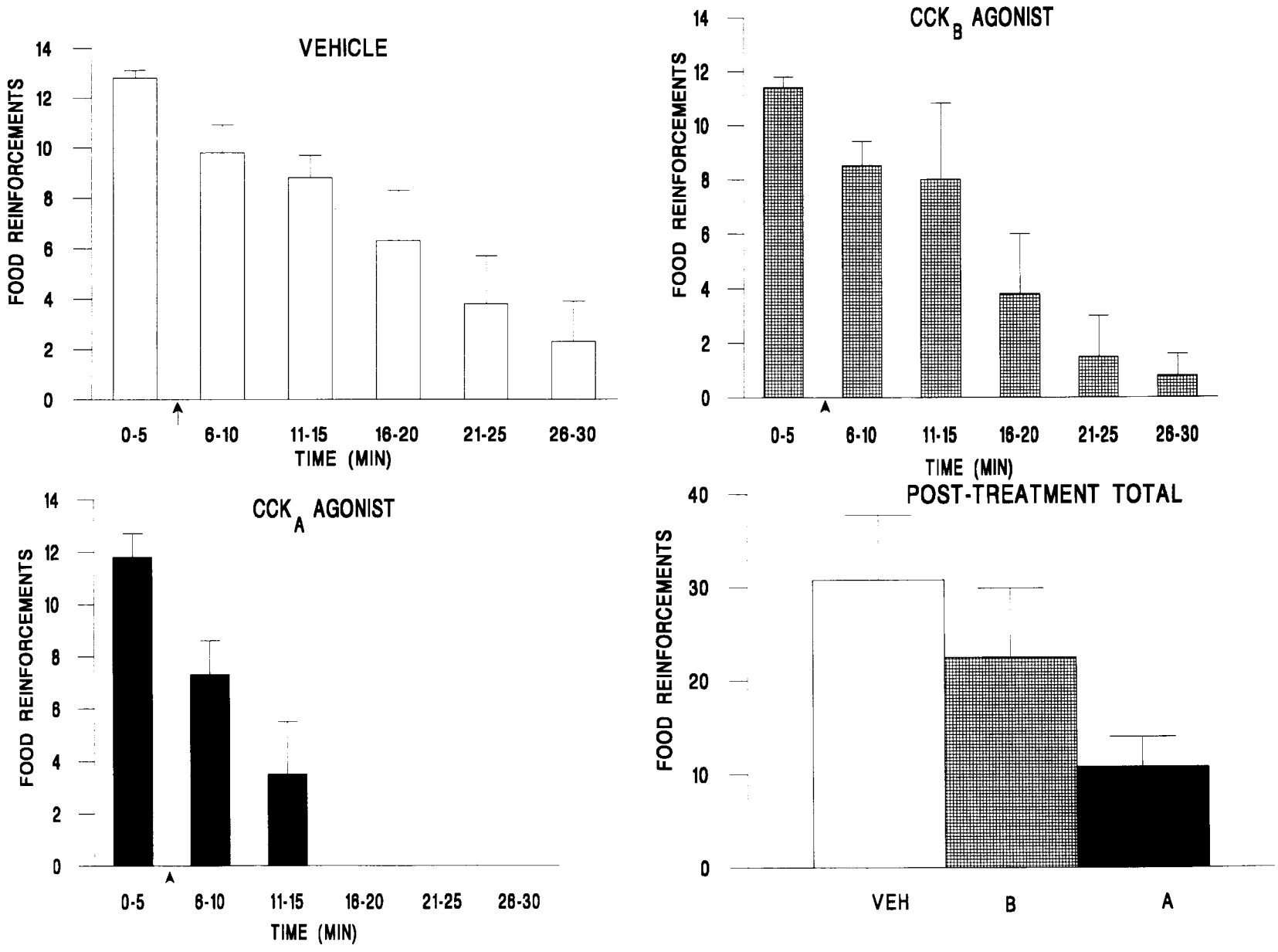

FIG. 2. Food reinforcements (mean ± SEM) obtained in 5-min periods before and after ICV injection (indicated by the arrow), and overall post-treatment totals, in pigs given vehicle or 5 μg CCK_A or CCK_B agonist in Experiment 1. Post-treatment totals were significantly different, $p < 0.02$ (see text for further details).

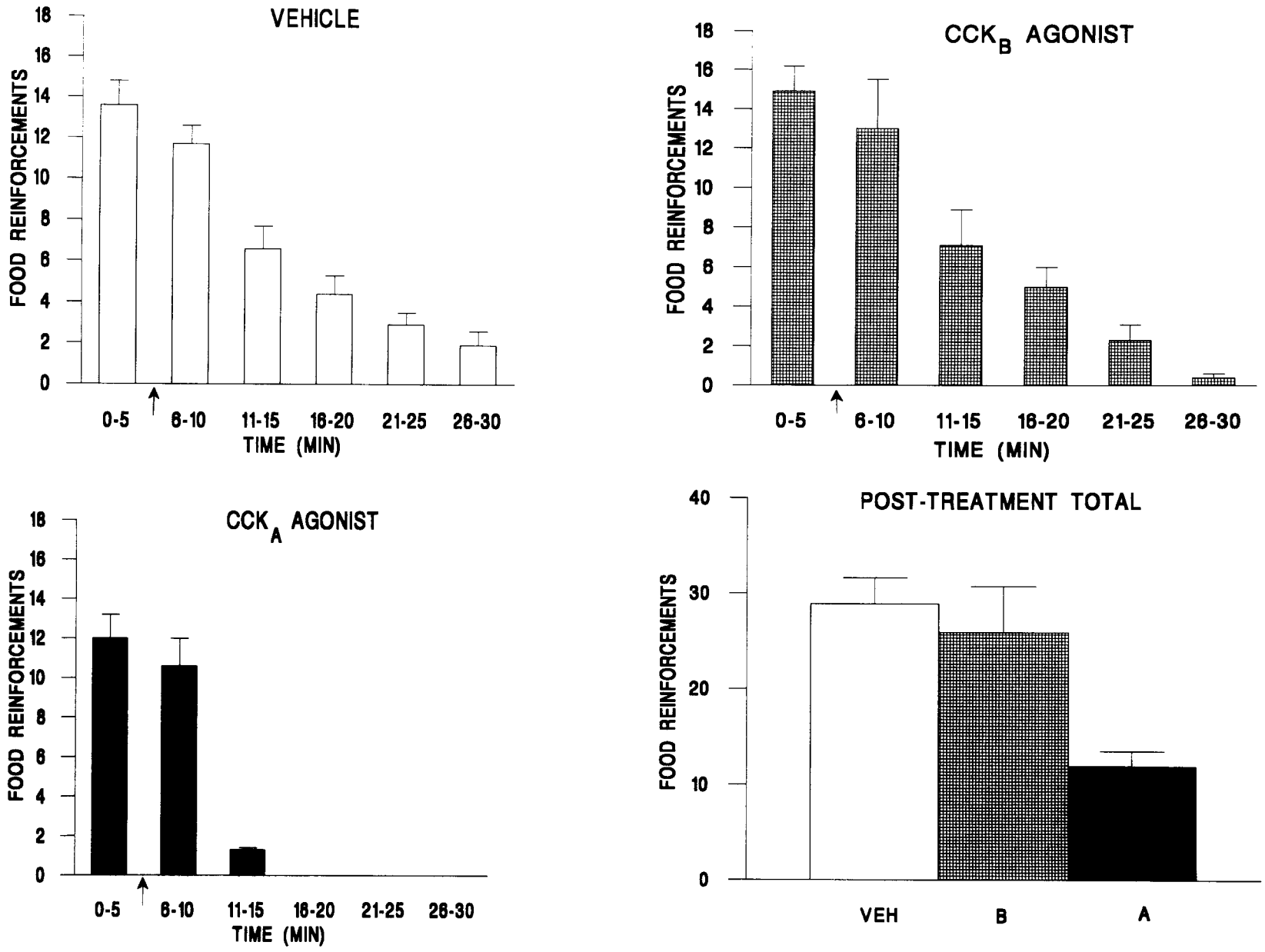

FIG. 3. Food reinforcements (mean ± SEM) obtained in 5-min periods before and after ICV injection (indicated by the arrow), and overall post-treatment totals, in pigs given vehicle or 1 μg CCK_A or CCK_B agonist in Experiment 1. Post-reatment totals were significantly different, $p < 0.01$ (see text for further details).

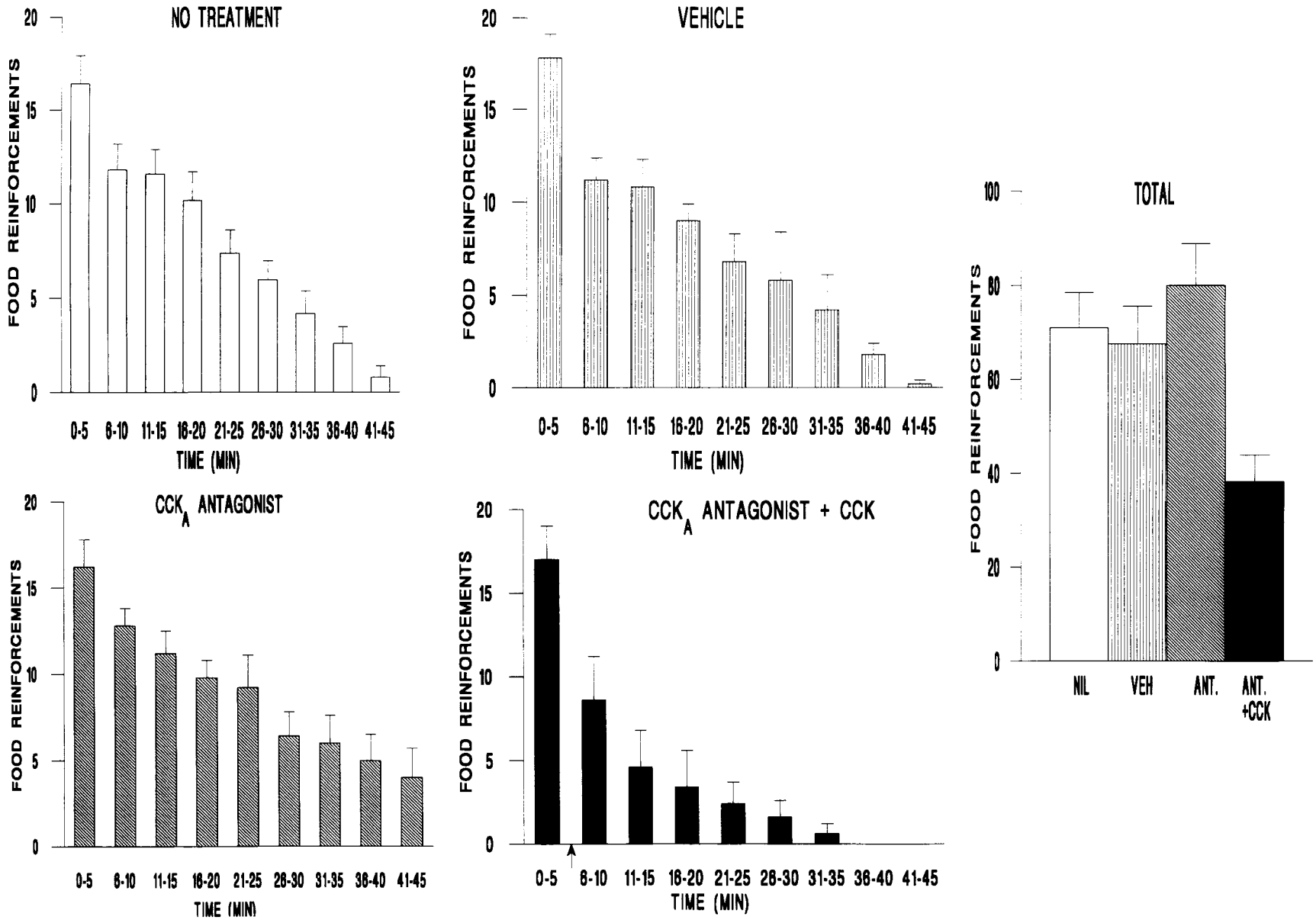

FIG. 4. Food reinforcements (mean ± SEM) obtained in 5-min periods, and overall totals in pigs receiving no treatment or pretreated with ICV injections of vehicle, 20 μg CCK_A antagonist, or CCK_A antagonist followed by ICV injection (indicated by the arrow) of 1 μg CCK in Experiment 2. Post-treatment totals were significantly different, $p < 0.001$ (see text for further details).

(12), a molar equivalent dose of A71623 (500 ng) would probably have comparable effects. Such considerations indicate that the inhibitory effects of exogenous CCK are more likely to be mediated by action at the CCK_A rather than the CCK_B receptor. Moreover, the reduction in meal size induced by pharmacologic doses of A63387 may represent a nonspecific aversive effect.

The stimulatory action of the CCK_A receptor antagonist devazepide on feeding in pigs (6,7,14) also supports the view that the CCK_A receptor may have some role in the control of normal ingestive behavior. However, another CCK_A receptor antagonist, A70104, does not increase appetite when given peripherally to pigs (7), although this could be due to its inability to cross the blood–brain barrier (3). When this hypothesis was tested in Experiment 2 by using a central route of administration, A70104 produced only a small (nonsignificant) increase in food consumption. However, this lack of response might be because, first, 17.5-h food-deprived pigs respond for food at a near-maximal rate and, second, because the dose of A70104 used (20 μg) was too low, as indicated by its inability to block the action of ICV CCK. This conclusion is supported by the results of a subsequent study (8), in which a higher ICV dose of A70104 (50 μg) not only increased feeding in 4-h deprived pigs but also blocked the inhibitory effect of the same ICV dose (1 μg) of CCK.

The present results are consistent with those reported in rodents and primates given A71623 and A63387 (3,9) and in agreement with those described for pigs after ICV administration of CCK_A and CCK_B antagonists (5). Together, these findings indicate that exogenous, and probably also endogenous, CCK influences feeding in the pig through an action at CCK_A receptor sites.

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