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BRIEF COMMUNICATION

Cyclic AMP and Central Noradrenaline Receptors: Failure to Activate Diencephalic Adrenergic Feeding Pathways

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HERBERG, L. J. AND D. N. STEPHENS. *Cyclic AMP and central noradrenaline receptors: failure to activate diencephalic adrenergic feeding pathways.* PHARMAC. BIOCHEM. BEHAV. 4(1) 107–110, 1976. – Intracranial injection of graded doses of dibutyryl 3′, 5′-cyclic adenosine monophosphoric acid (cAMP) at sites in the accumbens/stria terminalis nuclei of satiated rats elicited behavioural arousal and occasional convulsive episodes at higher doses, but failed to affect food consumption even in sites where injection of noradrenaline (65 nmol) consistently elicited increased feeding. Intracranial aminophylline (550 nmol) or dopamine (65 nmol) were also without effect on food consumption. This result does not support recent suggestions that cAMP serves as the second messenger in central noradrenergic motivational pathways.

Cyclic AMP Central noradrenaline receptors Adrenergeric feeding Dopamine Aminophylline

CYCLIC 3′, 5′-adenosine monophosphate (cAMP) is believed to serve as an intracellular second messenger for a number of hormones, neurohormones and neurotransmitters, including dopamine (DA) and adrenaline and for catecholamines (CA) acting on peripheral beta-receptors [26,35]. It is uncertain, however, whether cAMP is also the second messenger for noradrenaline (NA) at central synapses [10,24]. In support of this possibility is the finding that cerebellar Purkinje cells [32] and hippocampal units [29] depressed by microiontophoretic application of NA are affected similarly by iontophoretic cAMP. These findings have been interpreted as evidence that NA is yet another agent that acts on its receptors by triggering cAMP synthesis [32], and it has even been argued that the overriding factor determining the behavioural effects of brain NA is not the availability of NA but the degree to which subsynaptic adenyl cyclase responds to its presence by synthesising cAMP [33]. Several investigators, however, have failed to replicate the iontophoretic findings [14,22], and the correct interpretation of these findings is still warmly debated [3, 21, 30, 31]. Evidence that NA raises cAMP concentrations in brain slices [27], cell cultures [28] or whole brain [9] is similarly inconclusive: for although these changes can be partly prevented by either alpha- or beta-adrenergic blocking agents [24,27] they are not necessarily evidence of a synaptic action since NA causes cAMP to accumulate even in cells devoid of noradrenergic innervation [28].

Direct evidence of the role of cAMP at central synapses has been sought by comparing the behavioural effects of cAMP and NA applied directly to the brain. Small injections of NA [16] or NA-mimetics [4, 8, 34] into certain sites, or larger injections into the cerebral ventricles [23] elicit feeding behaviour in food-satiated rats which may be prevented by administration of alpha- but not beta-adrenergic blocking agents [4, 15, 34]. Using cAMP, usually in high or lethal doses, a number of investigators have demonstrated enhanced locomotor activity and emotional, sexual and other behavioural changes, including feeding behaviour, following intracranial administration in rats or cats [5, 6, 20]. The injections, however, were not administered specifically at brain sites known to contain NA receptors or to be particularly effective in eliciting adrenergic feeding, and it is not clear whether the feeding elicited after cAMP should be ascribed to a specific action by cAMP on NA-sensitive neurones, to general behavioural arousal, or (as suggested by one investigator [5]) to the known metabolic actions of cAMP. We have therefore examined the effect on feeding behaviour of cAMP injected in a range of doses through cannulas known to elicit adrenergic feeding. The same cannulas were used also to determine to what extent any feeding behaviour obtained with cAMP might be ascribed to DA receptors, and to investigate the effect of aminophylline, a xanthine derivative believed to cause enhanced intracellular levels of cAMP by preventing its breakdown by intracellular phosphodiesterase [11].

METHOD

Nine adult male Wistar rats were implanted with 22 ga. stainless steel cannulas aimed at the nucleus interstitialis striae terminalis, a structure rich in CA receptors [12], and effective in eliciting adrenergic feeding [8]. De Groot coordinates were A 6.4 to 8.4, 1.3 lateral, and 6.2 mm

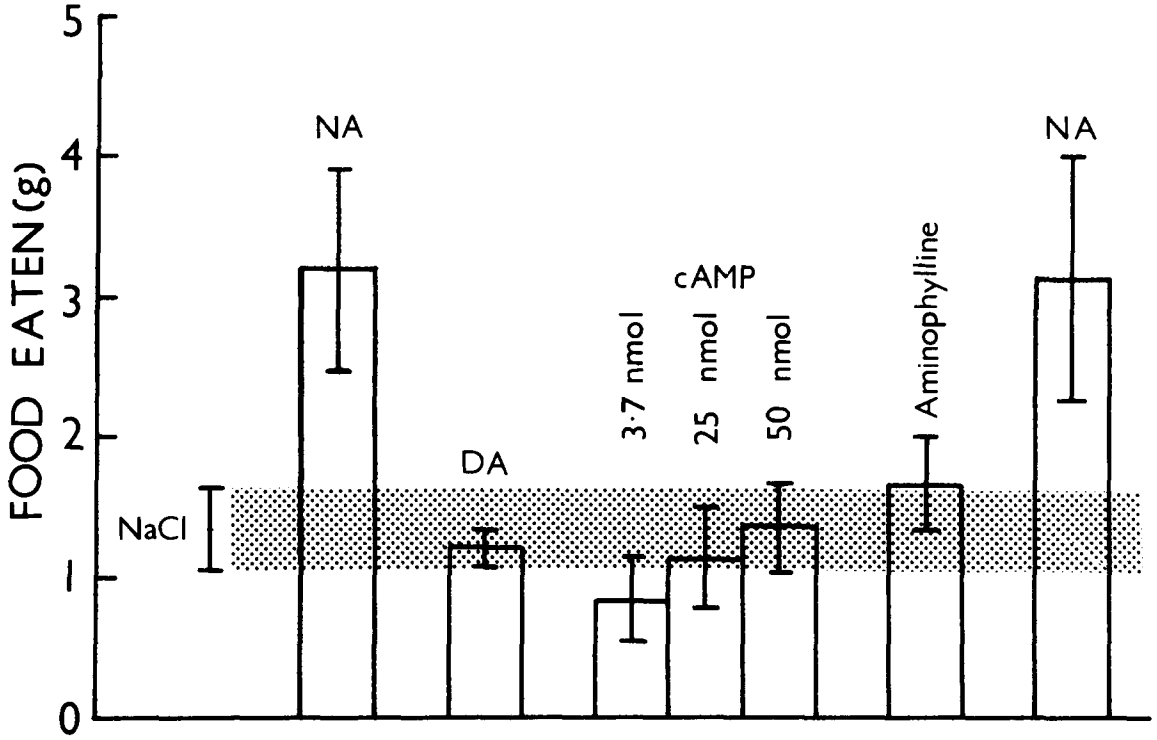

FIG. 1. Mean food intake during 1 hr test meals following intracranial injection of NaCl (155 nmol) (mean of 2 tests), NA (65 nmol) (mean of 2 tests), DA (65 nmol) (mean of two tests) dibutyryl cAMP (3.7, 25 and 50 nmol), aminophylline (550 nmol) and a further injection of NA (65 nmol). Scores were each derived from 8 rats except for the aminophylline and final NA tests where N = 7. Ranges shown are standard errors.

below the surface of the skull. After 2 weeks of recovery, the rats were given a series of intracranial injections each followed by a 1 hr feeding test according to procedures described previously [18]. Substances injected were noradrenaline bitartrate (65 nmol), dopamine HCl (65 nmol), aminophylline B.P. (Evans Medical) (550 nmol), and cyclic 3′, 5′-adenosine monophosphoric acid (3.7, 25 and 50 nmol) given as the dibutyryl salt (Sigma) to facilitate cell entry and to minimise its catabolism by phosphodiesterase. All solutions for injection were adjusted to a concentration of 155 mM by addition of NaCl, and given in a volume of 1.0 μl over a period of approximately 5 sec. Intervals of at least 72 hr were allowed between successive injections. Scores for NaCl, NA and DA were mean values obtained from tests administered in an ABCBCA sequence. The dose order for cAMP was 3.7, 50 and 25 nmol. At the end of the experiment animals were retested with NA and NaCl for a third time, and cannula sites were verified from photographic enlargements of 50 μm unstained frozen sections.

RESULTS

Figure 1 shows the mean weight of food pellets consumed in the 60 min after each of the drugs administered.

Noradrenaline caused significantly more feeding than NaCl (3.2 vs 1.3 g; $t = 2.9$, $d.f. = 7$, $p<0.05$) and 6 rats which exceeded a preset criterion [18] by consuming at least 1.0 g more food after NA than after NaCl were classified as feeders. Three rats were non-feeders (NA − NaCl < 1.0 g). Figure 1 includes scores of one non-feeder which died before receiving aminophylline. One feeder died after receiving only NA, and results from this rat were discarded. All but one of the 5 surviving feeders again exceeded criterion when retested with NA at the end of the experiment, while both the surviving non-feeders again failed to reach it.

Dopamine administration in rats classified as feeders was followed within 2–3 min by a brief burst of drinking followed by physical collapse during which the rat lay motionless and flaccid, responding only to strong stimuli. These effects did not occur in nonfeeders (nor was it described in previous investigations in which DA was injected in adrenergic feeding sites poor in DA receptors [4,34]. Recovery occurred within about 10 min but there was no significant change in the intake of either food pellets or water either in the feeders or in the group as a whole.

cAMP had no effect in any dose on food intake in either feeders or non-feeders (Friedman $\chi^2 = 0.8$; n = 8; N.S.). The two stronger doses induced obvious hyperactivity in all animals, and in three rats (2 feeders, 1 nonfeeder) this culminated in a brief convulsive seizure. Elevated rectal temperatures were recorded after convulsive seizures (39.0 to 39.5°C) but not in rats which did not suffer seizures.

Aminophylline scores were more than 1.0 g in excess of the corresponding saline scores in two rats (both feeders), but Fig. 1 shows that for the group as a whole there was no significant effect on the overall mean consumption, which was significantly less than consumption after NA (1.67 vs 3.5 g; $t = 3.3$, $d.f. = 6$, $p<0.05$).

Histology. All cannulas penetrated the lateral ventricle to terminate in the region of the posterior extremity of the nucleus accumbens at its junction with the bed nucleus of

the stria terminalis (de Groot planes A7.0–8.0). Some brains showed evidence of reflux into the ventricles along the cannula tracks, and the ventricles of 3 feeders were markedly dilated, indicating that the injected solutions may have spread considerably beyond the immediate site of injection.

DISCUSSION

Intracranial injection of cAMP in doses sufficient to affect gross behaviour failed to produce significant changes in food consumption, even though injections of NA caused more than a two-fold mean increase over saline control scores. Injections of aminophylline, designed to raise intracellular concentrations of endogenous cAMP by preventing its catabolism, were similarly ineffective. Thus cAMP is unlikely to serve as second messenger in the alpha-adrenergic feeding mechanism or in the ascending motivational pathways of which the feeding system is thought to be a constituent part [2, 7, 18]. The present findings do not argue against a role for cAMP at the adrenergic beta-receptor, but the weight of present evidence is against any significant role for central beta-receptors in motivation [1, 17, 19]. It becomes difficult, therefore, to account for the virtually perfect correlation, across rat strains, between the level of spontaneous motor activity and the degree to which NA stimulates the accumulation of brainstem cAMP [33]. In particular it is difficult to accept the suggestion that the level of spontaneous motor activity is determined by the sensitivity of brain adenyl cyclase to NA released at noradrenergic synapses. It would be interesting to learn, however, whether high sensitivity of adenyl cyclase to NA is associated with high sensitivity to other central transmitters, particularly DA and adrenaline which have recently been implicated in spontaneous motor activity [25] and arousal [13] respectively. If so, the correlation between motor activity and sensitivity to NA [33] could be properly regarded as incidental, not causal.

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