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Short-Term Feeding-Related Changes in Mediodorsal Hypothalamic Catecholamine Release

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KRUISSINK, N , J VAN DER GUGTEN AND J L SLANGEN *Short-term feeding-related changes in mediodorsal hypothalamic catecholamine release* PHARMACOL BIOCHEM BEHAV **24**(3) 575–579, 1986 —Release of noradrenaline (NA), adrenaline (A) and dopamine (DA) was measured in vivo per minute before and after food presentation in satiated rats that had a cannula in the mediodorsal hypothalamic area (MDH) Release of NA was significantly increased above baseline during a period of a few minutes in which feeding took place In the period following feeding there were peaks in the release of both NA and A In DA release no changes were observed Since a statistical correlation between release of NA or A and locomotor activity was not found, it is suggested that the increased release of NA and A is specifically related to food-motivated behavior

Catecholamine release Noradrenaline Adrenaline Dopamine Push-pull perfusion
Mediodorsal hypothalamus Feeding Locomotor activity

THERE is evidence that catecholamine (CA) neuron systems that innervate the hypothalamus are involved in feeding behavior The dorsal part of the catecholaminergic central tegmental tract has been ascribed a stimulatory function in feeding behavior, whereas the ventral part of the central tegmental tract has been attributed an inhibitory function, since lesions of the dorsal and ventral part result in, respectively, hypophagia and hyperphagia in rats [1,13] Fibers from the dorsal and ventral parts of the central tegmental tract predominantly have terminations in the mediodorsal and medioventral hypothalamic areas, respectively [14] Accordingly, lesions in the dorsomedial nucleus (DMN) produce hypophagia [3], especially in young rats, whereas lesions in the area surrounding the ventromedial nucleus cause hyperphagia [6] Furthermore, local injections of noradrenaline (NA) and adrenaline (A) into the mediodorsal hypothalamic area (MDH) of the rat can induce feeding [7, 11, 18], in contrast to dopamine (DA) and A injections into the lateral perifornical area which inhibit feeding [12]

More direct evidence for a role of brain catecholamines in feeding behavior has come from the finding that NA is released during circumstances that induce feeding Both concentrations and turnover of endogenous NA in the perifornical hypothalamus (PFH) and the DMN were shown to be elevated in groups of rats with high feeding rates compared with rats with a low feeding rate [21] When in the MDH of food-deprived rats release of NA was assessed in vivo by means of a push-pull perfusion technique, a significantly larger efflux of exogenous NA occurred during an interval of feeding than before feeding [15] In vivo release of both endogenous NA and DA from different hypothalamic sites was also measured simultaneously in rats that had continuous access to food [23] Comparison of 10 min perfusate fractions from before, during and after a period of spontaneous feeding revealed that there was a statistically significant increase in NA but not DA release from the MDH during feeding behavior No such an effect was observed in the subfornical hypothalamus

The aim of the present study was to distinguish hypothalamic CA release before, during and after the act of feeding by investigating the relationship between hypothalamic CA release, feeding and nonfeeding behaviors in a more detailed way than in former studies [15,23] To this end the release of NA, A and DA was measured each minute during a period of about 20 minutes, in which feeding was confined to a period of two minutes As a consequence CA release could be related to feeding and nonfeeding behavior and the release patterns before, during and after feeding could be compared statistically Local injections into the MDH of both NA and A lead to an enhancement of feeding behavior [7,18] The MDH is richly innervated by terminals of both noradrenergic and adrenergic neuron systems [9, 14, 22]. For these reasons, in the present study release of A was measured in addition to release of NA and DA Variability among subjects in CA release, due to properties of the push-pull perfusion technique, has been taken into account in the statistical analysis

METHOD

Animals

Six male Wistar rats weighing approximately 250 grams were used They were housed individually in macrolon home cages The animals had free access to water Standard lab-

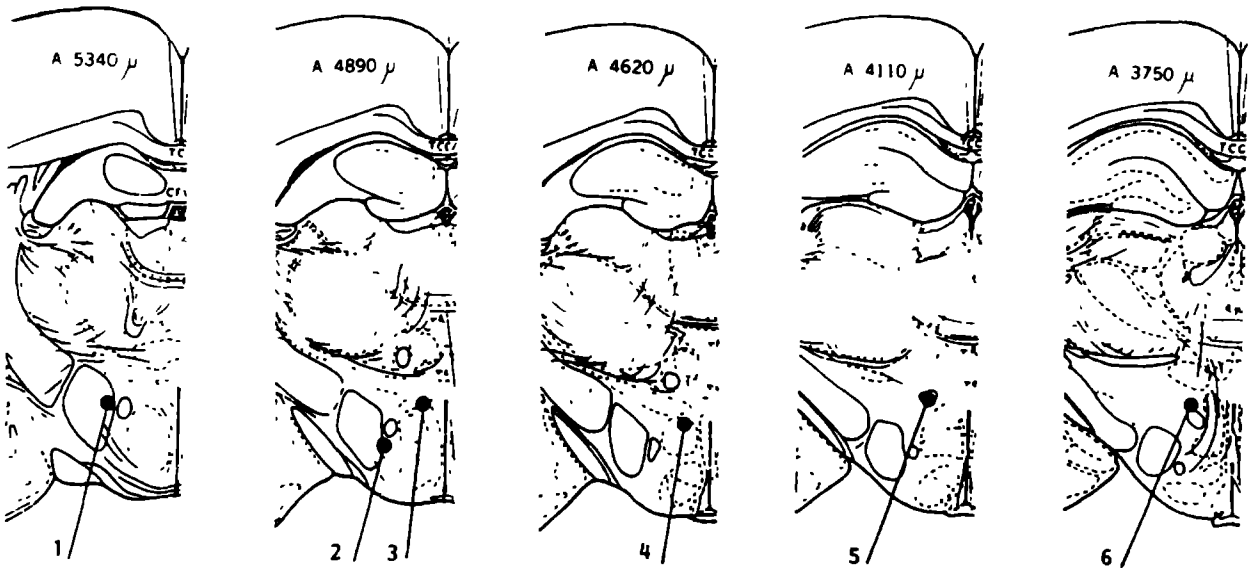

FIG 1 Diagrammatic representation of hypothalamic perfusion sites The loci are projected on frontal sections according to the atlas of Konig and Klippel [10] Inferior numbers refer to individual rats

oratory food (Muracon I) was available ad lib unless otherwise stated Lights were on from 08 00 a m to 08 00 p m Room temperature was kept at 22–24°C

Surgery

The outer cannula of a concentric perfusion system was stereotaxically implanted in the brain of a rat under anesthesia and fixed to the skull as previously described [23] The intended site of perfusion was the mediodorsal hypothalamic area (MDH)

Pretreatment

Following the operation the rats were allowed at least seven days for recovery When from changes in body weights it was concluded that growth following the operation was normal, food intake was recorded continuously during one night in the experimental cage (25×25×25 cm), equipped with a grid floor and a feedometer system that made Noyes pellets (45 mg) continuously available From the recordings it was decided whether the rats had retained a normal nocturnal feeding pattern In addition each rat was allowed to stay once in the experimental cage between 02 00 and 04 00 p m and to eat approximately ten Noyes pellets after each 15 min interval

Push-Pull Perfusion

Push-pull perfusion took place between 02 00 and 04 00 p m in unanesthetized rats, freely moving in the experimental cage Prior to an experiment the inflow and outflow nipples on the removable inner part of the push-pull cannula system [23] were connected via polypropylene tubing (internal diameter 0 30 mm) to pieces of silicon rubber tubing (internal diameter 0.65 mm), mounted on a two-way swivel (internal volume of the outflow part was 1 5 μl) and leading to two peristaltic pumps (LKB, Varioperpex® II, 2120) The two-way swivel served to prevent that the in- en outflow tubing became twisted or was grasped by the animal A small pressure transducer (Entran Devices, NJ) was connected to the inflow tubing in order to check pressure in the course of a perfusion experiment The complete tubing system was filled with perfusion fluid of the following composition 151 mM NaCl, 2 4 mM KCl, 1 1 mM $CaCl_2$ and 4 4 mM reduced glutathione, adjusted to pH 7 4 The loose end of the inflow tubing was immersed in a beaker containing the perfusion fluid, and the inner cannula was inserted into the chronically implanted outer cannula The total volume of the tubing system from the tip of the cannula to the site of collection amounted to 120 μl, approximately After the start of the experiment, the velocity of the perfusion fluid in the outflow tubing was measured in order to determine the delay in the collection of the neurotransmitters relative to the actual time of release

The tip of the inner cannula protruded 0 3 mm beyond the tip of the outer cannula The perfusion was carried out at a rate of 20 μl per minute for approximately one hour With each animal one perfusion experiment was carried out

In the first half hour the perfusate fractions were pooled Thereafter perfusate was collected continuously in 1-min fractions at the end of the outflow tubing, during approximately half an hour After collection of a perfusate fraction 40 μl of a dilutive solution was added in order to prevent degradation of the CA It had the following composition 0 3 N $HClO_4$, 4 0 mM disodium EDTA and 5 4 mM reduced glutathione The samples were stored at −15°C for two weeks at most

During the second half hour of a session food (Noyes pellets) was offered to the animal When a rat had fed during two minutes, feeding was interrupted by the experimenter by withholding further food presentation The animal had free access to water from a drinking tube attached to the cage Feeding was registered automatically In addition, the following behavioral elements were recorded manually walking, rearing, grooming, sitting, lying immobile and drinking The occurrence of an element was scored for every 15 sec period Locomotor activity was scored from 0–4, depending

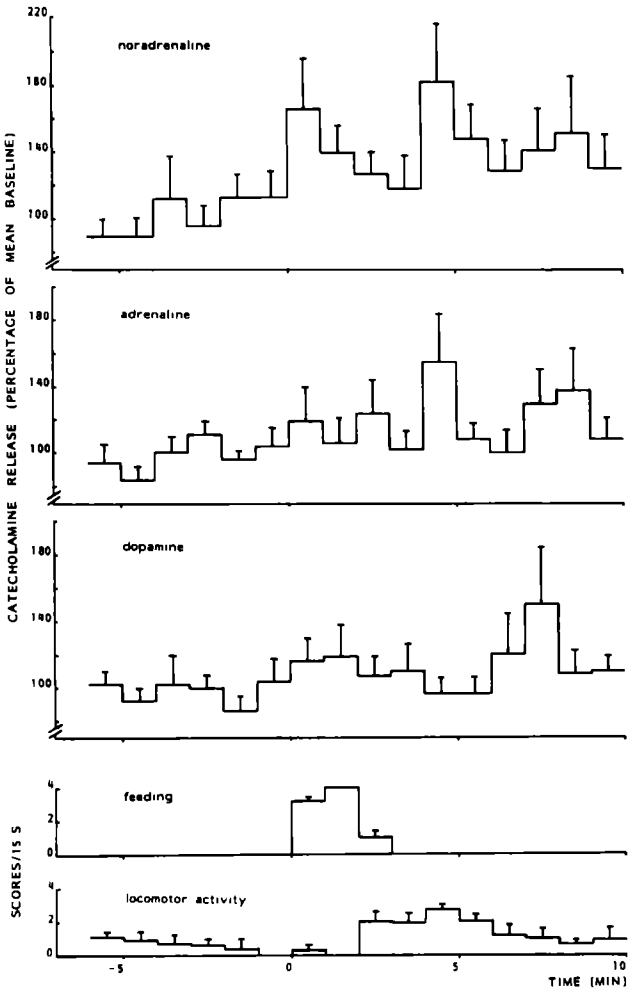

FIG 2 CA release before, during and after feeding in response to limited food presentation Graphs from top to bottom represent means and S E M (N=6) for NA, A and DA release, and for incidence of feeding and locomotor activity in 15-sec periods Feeding began at $t=0$, food was presented until feeding had occurred for 2 min

on the number of 15 sec periods per minute in which walking and/or rearing occurred

Histology

At the end of a push-pull perfusion experiment the animal was anesthetized with an overdose of sodium pentobarbital and perfused with 0 9% NaCl and 10% formalin successively The brain was frozen and frontal serial sections of 100 μm were cut at an angle of about 15° to the tract of the cannula system Sections were stained with thionin The site of perfusion was microscopically localized with reference to the atlas of Konig and Klippel [10] and identified as a point 0 2 mm dorsal to the bottom of the tract and 0 3 mm rostral to its caudal extension

Catecholamine Assay

Diluted perfusate fractions were thawed and duplicate 20 μl samples were transferred into conical glass tubes Samples and blanks were analyzed according to an enzyme-radiochemical method [22] Blanks consisted of diluted and pooled perfusate fractions to which calcium instead of magnesium was added Each assay series also included internal CA standards in pooled perfusate fractions. The CA were converted into their [^{3}H] methoxy derivatives by incubation with S-adenosyl-L-[methyl-^{3}H] methionine (New England Nuclear, Boston, 60–80 Ci/mmole) in the presence of catechol-O-methyltransferase The labeled products were isolated by organic extraction and paperchromatography, radio-activity was counted in a liquid scintillation analyser (Philips, Holland) CA release data were calculated from net dpm values for samples and internal standards and were expressed as pg/min

Statistical Analysis

A total of eighteen one-minute perfusate samples from each perfusion experiment was assayed for CA The six one-minute values preceding the offering of food were considered the baseline values Statistical independence of the data was tested for by calculating serial autocorrelations with different time lags [5], per rat and per CA, separately, for a total time series of sixteen minutes, the baseline period, the period after food offering and the period after feeding

The effects of condition (two levels baseline period and feeding period or post-feeding period), subjects (six levels) and their interaction on CA release per minute were analyzed with two-way uni- and multivariate analysis of variance (ANOVA and MANOVA, [4]) The effect of these variables on the release of NA, A and DA separately was tested with ANOVA, the effect on the release of NA, A and DA in combination was tested with MANOVA Two minutes of feeding were taken into account for each rat In order to detect a general trend in CA release in the post-feeding period the data of seven post-feeding minutes were compared with ANOVA (randomized blocks analysis with repeated measures on subjects)

In addition, with the above ANOVA and MANOVA analyses, CA release during the baseline period was compared with CA release during the first two minutes after food presentation, irrespective of whether the rats were feeding or not

Locomotor activity scores were analyzed by means of ANOVA, with factors condition (two levels baseline period versus the post-feeding period), subjects and their interaction In addition, the possible statistical correlation between CA release and locomotor activity was tested with point-biserial correlation [8] and Pearson correlation between CA release and scores of locomotor activity per minute. For the calculation of point-biserial correlation, the minutes were categorized as containing locomotor activity when there was locomotor activity in at least half a minute Both correlation coefficients were calculated for the measurements of each CA and for each rat separately They were calculated for the total time series and for the post-feeding period separately The criterion for statistical significance was considered to be $p<0$ 05

RESULTS

Histological analysis revealed that the cannulas had reached the intended area in and around the dorsomedial nucleus of the hypothalamus (see Fig 1) In two rats the tip

of the cannula was in the perifornical area (PFH) In one rat it was in the paraventricular nucleus (PVN) In the remaining three rats the tip of the cannula was in or near the DMN The width of the cannula tract did not exceed the 0 65 mm diameter of the outer cannula by more than 0 15 mm

The mean patterns of CA release are shown in Fig 2 The autocorrelation coefficients of the CA measurements over time were not significant and thus the values of CA release in the successive minutes were considered to be statistically independent

During the baseline period the mean release for respectively NA, A and DA was 5 8 pg/min, 6 2 pg/min and 3 6 pg/min During feeding the mean increase in CA release was 53% for NA, 13% for A and 18% for DA MANOVA revealed that there was a multi-variate significant difference between baseline period and feeding period in the release of NA, A and DA in combination $F(3,34)=3\ 58$, $p<0\ 05$ However, the univariate F ratios make clear that only NA release was significantly elevated during feeding $F(1,36)=7\ 28$, $p<0\ 01$ No significant changes in NA release were observed in connection with food presentation per se During the first two minutes after food presentation the mean NA release was increased by only 27% over baseline levels

Figure 2 shows that also in the post-feeding period the level of CA release was increased compared with the baseline level The increments amounted to 43%, 20% and 13% for NA, A and DA, respectively, and were significantly different from baseline release for NA and A $F(NA)(1,66)=4\ 01$, $p<0\ 05$, $F(A)(1,66)=4\ 09$, $p<0\ 05$ Within this post-feeding period no significant trends in CA release over time and subjects were found

The rats differed significantly from each other in mean NA, A and DA release in the total test period ($p<0\ 0001$) For the feeding period, there was no significant interaction effect of the factors condition and subjects on NA and DA release, i e , the individual NA responses observed did not depend on brain site In contrast, such an interaction did exist for A release $F(5,36)=2\ 64$, $p<0\ 05$ For the post-feeding period, there was a significant interaction effect on DA release only $F(5,66)=2\ 99$, $p<0\ 05$

In the baseline period walking, rearing, grooming, sitting and lying immobile occurred After food presentation, three animals fed within 15 sec and the remaining animals fed within two minutes Feeding continued until food presentation was withheld In the post-feeding period locomotor activity (walking and rearing) was significantly increased in comparison with the baseline period $F(1,5)=7\ 36$, $p<0\ 01$ Figure 2 suggests that in individual rats, the peaks of CA release and locomotor activity in the post-feeding period are correlated For the total time series a correlation was found in one rat, namely between NA release and locomotor activity ($r(pbis)=0\ 53$, $t=2\ 17$, $df=12$, $p<0\ 05$, Pearson, $r=0\ 53$, $df=12$, $p<0\ 05$) For the post-feeding period also in only one (other) rat a significant positive correlation was found between NA release and locomotor activity ($r(pbis)=0\ 63$, $t=1\ 98$, $df=6$, $p<0\ 05$, Pearson, $r=0\ 74$, $df=6$, $p<0\ 05$) For the other rats, for rats pooled together and for release of A and DA the correlations were not significant or sometimes significantly negative

DISCUSSION

The results of the present study demonstrate that short-term changes in CA release from the rat mediodorsal hypothalamus occur in connection with a short period of food presentation In particular, NA release was enhanced during a 2 min feeding period, whereas increases in both NA and A release were evident immediately after feeding The increased release of NA during and after feeding may reflect increased activity of noradrenergic nerve fibers with terminals in the MDH

The increased NA release, observed in the post-feeding period, was not related to locomotor activity It may be related to the various metabolic changes that occur following a meal For instance, the amount of circulating insulin is increased biphasically during and after feeding [19,20] Actually, hypothalamic NA turnover was shown to be enhanced by glucoprivation induced by peripheral injections of insulin [2] Moreover, in some regions of the hypothalamus, the local application of insulin led to a delayed increase in the apparent activity of CA neurons [16] However, it is not clear whether release of pancreatic insulin into the circulation could be the direct cause of elevated hypothalamic NA release Alternatively, a rise in mediodorsal hypothalamic NA release might represent a neural signal which leads to metabolic changes related to feeding, since areas within the medial hypothalamus have been shown to exert influences on peripheral metabolic processes [3]

During the post-feeding period, A release was also increased This result suggests a role of A in food-motivated behavior, and may be related to the observation that feeding behavior can be induced by local MDH injections of A as well as NA [7, 11, 18] The observation that DA release was not significantly elevated during and after feeding is in agreement with the fact that local injection of DA into the MDH does not induce feeding behavior [18]

The present results do not confirm the view that the smell of food is sufficient for the occurrence of the feeding-related increase in NA release, as has been suggested by the finding that the efflux of exogenous NA was increased during olfactory stimulation in food-deprived rats [17] In our study, food presentation per se, which would imply olfactory stimulation, did not lead to a significant increase in NA release However, it remains possible that an effect of olfactory stimulation on NA release depends on deprivation level

Individual rats differed significantly in the extent of their CA release throughout the whole perfusion period These differences might be attributed to artifacts in the perfusion technique or to differences in perfusion sites However, the observation that there were significant effects of interaction between condition and subjects on mediodorsal hypothalamic A and DA release, but not NA release, suggests that NA neurotransmission has the strongest relationship with food-motivated behavior and is influenced least by inter-individual and inter-experimental variations The interaction effect on A release, as well as the absence of an overall increase in A release during feeding, may be explained by the fact that two out of six subjects had a cannula in the PFH It has been shown that A concentrations in the DMN were different for groups of rats with different feeding rates, whereas A levels in the PFH were not [21] In addition, A injections into the lateral PFH can inhibit feeding behavior [12] These findings corroborate the view that the MDH contains functionally different populations of A nerve terminals

From histological analysis it can be concluded that the sites of perfusion were in hypothalamic regions that are known to be richly innervated by noradrenergic and adrenergic nerve terminals [14,22] In addition, CA terminals in these regions have been implicated in feeding behavior (i e , PFH [18], PVN [11], DMN [3]) One might question whether

the effects observed in the present study are specifically confined to the MDH Obviously, the perfusion technique might impose limitations on the localization of the area involved, due to the potential for tissue destruction However, the width of the lesion after the perfusion under these relatively mild and controlled conditions hardly exceeded the diameter of the cannula tract in non-perfused animals (see the Results section) Moreover, in two other studies (Kruissink, Van der Gugten and Slangen, to be published, and [23]), rats were normally responsive to NA injections into the MDH after they had been subjected to push-pull perfusion for a much longer time than in the present study The anatomical specificity of the NA responses observed follows from similar perfusion experiments on two rats that had a cannula in the medioventral hypothalamus In both animals, NA release was not enhanced during and after feeding in comparison with the baseline Therefore, the MDH can be considered to be a functionally distinct area of the hypothalamus as to the involvement of NA terminals in feeding behavior

By measuring CA release per minute it was possible to observe short-term changes in CA release in relation to feeding These changes were shown to be specifically related to feeding since no relationship was found with locomotor activity For these reasons, it is concluded that the present method is a valuable approach to the study of correlations between CA release and different behaviors

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