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Release of Endogenous Catecholamines from Rat Hypothalamus in vivo Related to Feeding and Other Behaviors[1]

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VAN DER GUGTEN, J. AND J. L. SLANGEN. *Release of endogenous catecholamines from rat hypothalamus in vivo related to feeding and other behaviors.* PHARMAC. BIOCHEM. BEHAV. 7(3) 211–219, 1977. – The release of endogenous noradrenaline (NA) and dopamine (DA) from various sites in the hypothalamus was determined in unanesthetized, freely moving rats by means of a push-pull perfusion technique in combination with a sensitive radiochemical assay. The perfusate was collected continuously over 10-min periods for 4 to 8 hr. Patterns of feeding, drinking, grooming, and locomotor activity were recorded during the perfusion experiments. Release patterns and behavior recordings were analysed by tests of serial correlation. Although significant variations in catecholamine release over time were observed, they did not reflect a fixed autonomic periodicity. Release from the medial hypothalamus was measured in rats which had been deprived of food for 16 hr and were subsequently given free access to food. Before food presentation, mean NA release over 10-min periods was consistently higher than during satiety. Mean NA and DA release over 10-min periods before, during and after the occurrence of feeding, drinking or grooming were calculated for the dorsomedial, perifornical, subfornical, and anterolateral hypothalamus of freely feeding rats. During feeding, NA (but not DA) release from the dorsomedial and perifornical areas was significantly elevated (40–50%) when compared with pre- or postfeeding values. An increase in catecholamine release was not observed during drinking or grooming. In addition, NA release from the anterolateral area correlated with locomotor activity. The enhancement of NA release from the perifornical area during feeding is considered to be specific as no change in release was observed even during the vigorous drinking response induced by angiotensin.

Catecholamines Noradrenaline Dopamine Release Push-pull perfusion Hypothalamus Perifornical region Dorsomedial area Feeding Drinking Grooming Locomotor activity

IN ADDITION to pharmacological procedures [2, 3, 6, 10, 13, 14, 23, 24], neurochemical methods have been employed to study the involvement of central catecholamine (CA) neuron systems in the regulation of food intake in the rat. The measurement of chemical parameters in large brain parts in vivo [8] and in vitro [27] revealed changes in activity of hypothalamic CA neurons possibly related to alterations in feeding conditions. Regional changes in hypothalamic CA metabolism were also observed; the activity of noradrenaline (NA) and adrenaline neurons in the dorsomedial nucleus as well as NA neurons in the perifornical region appeared to be enhanced in rats showing relatively high feeding rate [25].

Moreover, the washout of radioactivity from the area adjacent to the third ventricle as determined after the injection of [^{14}C]-NA was enhanced during feeding in previously food deprived rats [15]. The efflux of radioactive material, however, is considered to be a poor index of neuronal transmitter release. The labeled CA which must be applied in a high dose is progressively metabolized during the perfusion [16,17] and nonspecific release may occur [17]. Furthermore, the time-dependent decline in the radioactivity of the effluent does not allow the estimation of absolute amounts of released CA.

The development of enzyme-radiochemical assay procedures made it possible to determine in vivo release of endogenous CA from brain tissue. Release of NA and dopamine (DA) was demonstrated at a large surface of cerebral cortex of the cat [22] whereas DA release was also observed in the cat caudate nucleus and nucleus accumbens [1] which are very rich in this compound.

We used a sensitive micro-method for the simultaneous

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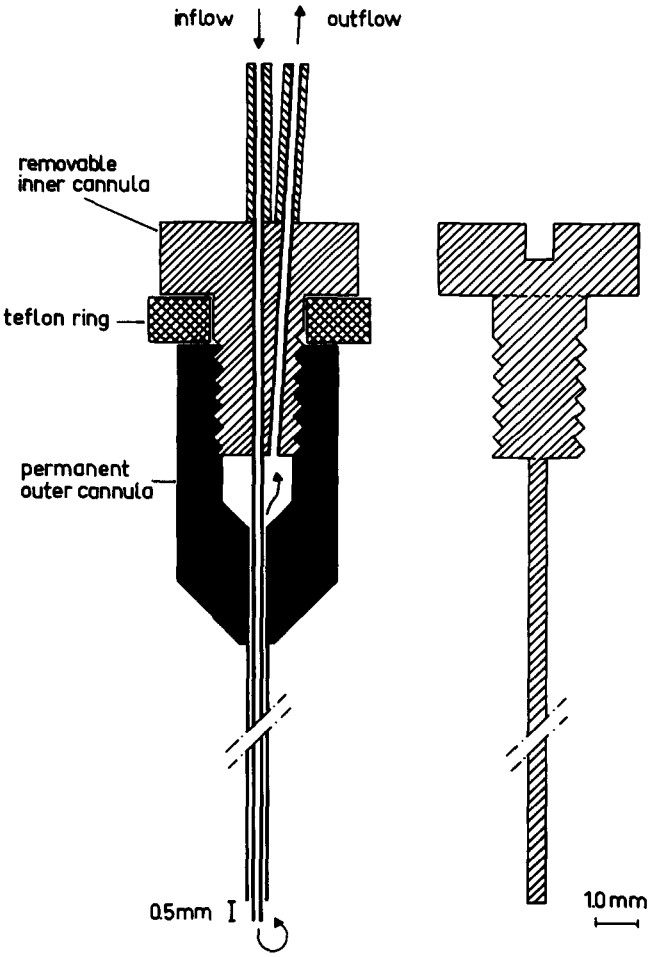

FIG. 1. Schematic representation of the push-pull cannula system. The needle of the permanent outer cannula had an outer diameter of 0.65 mm and an inner diameter of 0.40 mm; the needle of the removable inner cannula had an outer diameter of 0.30 mm. The total volume of the outflow part of the system was 7.5 μl. The protective wire stylette is represented on the right.

assay of NA, DA and adrenaline [26], in combination with a push-pull perfusion technique, for the continuous measurement of endogenous NA and DA release from areas within the hypothalamus of unanesthetized, freely moving rats. The data were analysed in relation to patterns of feeding, drinking and grooming as well as locomotor activity which were continuously recorded during the perfusion experiments. This approach allowed evaluation of the specificity of correlations between the neurochemical and behavioral parameters.

METHOD

Animals

Twenty-five male Wistar rats weighing approximately 250 g were used. They were housed individually in wire-metal home cages. The animals had always free access to water. Standard laboratory food (Muracon I) was available ad lib. unless otherwise stated. Lights were off from 19.00 to 7.00 hr. Room temperature was kept at 22–24° C.

Surgery

The outer cannula of a concentric perfusion system (Fig. 1) was stereotaxically implanted in the brain of a rat which had been anesthetized with Hypnorm (Philips-Duphar), containing 10 mg fluanisone and 0.2 mg fentanyl per ml, given subcutaneously in a dose of 1 ml/kg. The stainless steel needle of this cannula was lowered into the brain according to the Pellegrino and Cushman coordinate system [19] until the tip rested at 0.25 mm above the intended site of perfusion within the hypothalamus. Sites medial to the fornix were approached at an angle of 5° to the midsagittal plane in order to avoid damage to the wall of the third ventricle. The cannula was fixed in place with acrylic cement which was poured around the hub and anchored with four stainless steel screws in the skull. A removable wire stylette attached to a threaded cap (Fig. 1) was then inserted into the cannula so that the tip of the wire was exactly flush with the tip of the cannula.

Five rats were implanted with an additional cannula aimed at the lateral ventricle. This cannula was constructed from polypropylene tubing (internal diameter 0.50 mm) and was used for intraventricular injections. Stereotaxic coordinates [19] of the tip of the cannula were the anterior-posterior level of bregma, 1.5 mm lateral to the midline and 3.5 mm ventral to the dura. The rats were allowed at least 7 days of recovery before perfusions were carried out.

Push–Pull Perfusion

For the perfusion in an unanesthetized rat, a Plexiglas experimental cage was used. The cage had a height of 20 cm and was equipped with a grid floor of 20 × 10 cm^2. One of the large walls inclined at an angle of 70° to the floor, which forced the animal to turn back only by the opposite wall and thus prevented twisting of inflow and outflow tubing during the continuous perfusion. Food pellets (Noyes, 300 mg) could be offered in a trough and water was available from a drinking tube attached to the cage. Before perfusion experiments were performed, each animal was habituated to the experimental situation by being placed in the cage for periods of at least 60 min on three separate days.

Prior to an experiment, the inflow and outflow nipples on the isolated inner part of the push-pull cannula system (Fig. 1) were connected via polypropylene tubing (internal diameter 0.50 mm) to pieces of silicon rubber tubing (internal diameter 0.65 mm) mounted in two peristaltic pumps (LKB, Varioperpex 12000). The complete tubing system was then filled with perfusion fluid (Merlis' solution) of the following composition: 119.0 mM NaCl, 3.3 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.5 mM Na_2HPO_4, 21.0 mM $NaHCO_3$, 3.4 mM glucose, 2.2 mM urea; pH 7.4. The loose end of the inflow tubing was immersed in a beaker containing this solution and the inner cannula was screwed into a chronically implanted outer cannula. The tip of the inner cannula protruded 0.5 mm beyond the tip of the outer cannula. The perfusion was carried out at a rate of exactly 10 μl/min for 4 to 8 hr.

Perfusate was collected continuously in 10-min fractions at the end of the outflow tubing. The total volume of the tubing from the tip of the cannula to the site of collection amounted to 200 μl. Therefore, a delay of exactly 20 min in the collection of the perfusate relative to the actual release was taken into account. Immediately after a 10-min fraction was obtained, 10 μl of 27 mM disodium ethylenediaminetetraacetic acid was added in order to prevent degradation of the CA. The volume of each fraction was

measured; no significant deviations in perfusion rate were detected. The samples were stored at −15°. Fractions collected during the first 30 min of an experiment were used for the preparation of internal CA standards.

The rat's behavior was continuously observed during the perfusion. Onset time and duration of feeding, drinking, and grooming were recorded. Locomotor activity defined as the number of rearings and turnings was scored. No abnormal behavior was observed during or immediately after the perfusion. A maximum of three perfusion experiments was performed with one animal.

In some experiments, 200 ng of the synthetic octapeptide (valine^5)-angiotensin II amide (Hypertensin, CIBA) was administered at intervals of at least 60 min. This compound was injected through the ventricular cannula in a volume of 2 μl while the animal was left in the cage. Other drugs were administered intraperitoneally (IP).

Catecholamine Assay

Perfusate fractions were thawed and triplicate 20-μl samples were transferred into conical glass tubes. Samples and blanks were analysed according to an enzyme-radiochemical method [26] in a random order. Blanks consisted of Merlis' solution to which was added 0.1 volume of 27 mM ethylenediaminetetraacetic acid. Each assay series also included internal CA standards in pooled perfusate fractions and external standards added to blanks. Internal standards were exposed to room temperature for 20 min. The CA were converted into their [^{3}H]-methoxy derivatives by incubation with S-adenosyl-L-[methyl-^{3}H] methionine (The Radiochemical Centre, Amersham; 8–10 Ci/mmole) in the presence of catechol-O-methyltransferase. The labeled products were isolated by means of organic extraction and paperchromatography. CA concentrations in the perfusate fractions were calculated on the basis of values obtained for internal CA standards. The mean recovery of internal standards amounted to 76% of the yield of external standards. Cross contamination, although less than 1%, was corrected for. The sensitivity of the assay was approximately 10 pg. Although the amounts of CA in the samples were close to this limit, the analysis of triplicate samples allowed a reliable determination of CA release. A particular CA was considered to be not detectable when the mean value obtained for the perfusate fractions was not significantly different from that found for blanks.

Noradrenaline Tests

The construction of the push-pull cannula system offered the possibility of micro-injections at the perfusion site. After a series of perfusion experiments, each animal was tested for the effect of exogenous NA on food intake. The aim of these tests was to establish whether a normal sensitivity to NA existed even after repeated perfusions, and to determine the correlation between the action of NA at a particular site and the local CA release pattern. The tests were always carried out between 10:00 and 16:00 hr. After a rat had been taken from its home cage, the protective stylette was removed from the permanent cannula and subsequently replaced. The animal was then placed in a wire-metal test cage where it had access to a known amount of food for 60 min. After this period, the amount of consumed food was determined and 20 nmoles of L–NA (bitartrate salt, Sigma) were injected via the cannula in a volume of 1 μl. The tip of the micro-injection needle protruded 0.25 mm beyond the bottom of the cannula. The animal was replaced in the test cage and food intake over 60 min was again measured. The effect of NA on food intake is expressed as the difference between the amount of food consumed during the hour after the injection and intake during the hour before. Three NA tests were performed with each rat at intervals of at least 2 days.

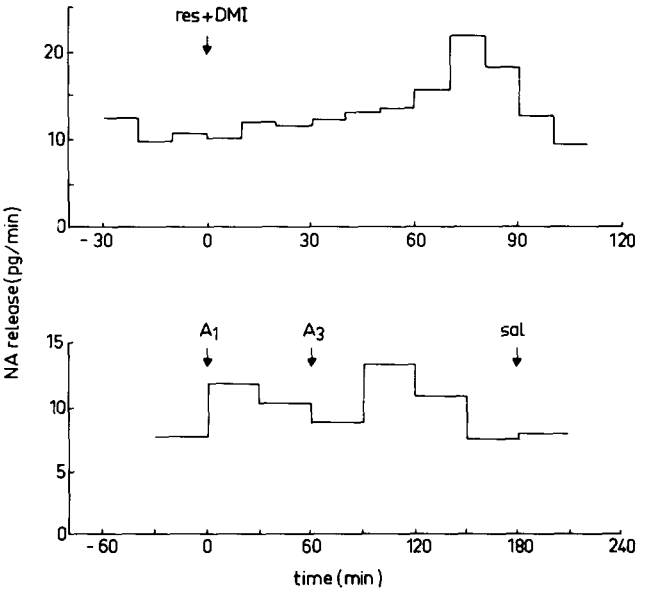

FIG. 2. Effects of drugs on hypothalamic NA release. Reserpine (res, 5 mg/kg IP) in combination with desmethylimipramine (DMI, 25 mg/kg IP) was administered to an unanesthetized rat (upper panel). D-Amphetamine (A_1, 1 mg/kg; A_3, 3 mg/kg IP) or saline (sal) was injected in an animal anesthetized with urethane (1.25 g/kg IP) (lower panel). Arrows indicate the times of injection.

Histology

After the experiments and NA tests, each animal was anesthetized with an overdose of sodium pentobarbital and successively perfused with 0.9% NaCl and 10% Formalin. 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 localized by direct projection of the sections onto the atlas of König and Klippel [12] and identified as a point 0.3 mm dorsal to the bottom of the tract and 0.2 mm rostral to its caudal extension. The diameter of the tract at the level of the perfusion site was 0.4 mm at most.

Statistical Analysis

Each time series consisting of CA release data or locomotor activity scores was analysed by tests of serial correlation in order to investigate the statistical dependence of the values. Serial correlation coefficients (SCCs) were calculated by means of a general-purpose electronic computer (CDC Cyber). The correlation coefficient (r_k) at the time lag of k time units (10 min) is expressed by the equation

$$r_k = \frac{n(\Sigma x_i y_{i+k}) - \Sigma x_i \Sigma y_{i+k}}{\sqrt{\left[\left\{n\Sigma x_i^2 - (\Sigma x_i)^2\right\}\left\{n\Sigma y_{i+k}^2 - (\Sigma y_{i+k})^2\right\}\right]}}$$

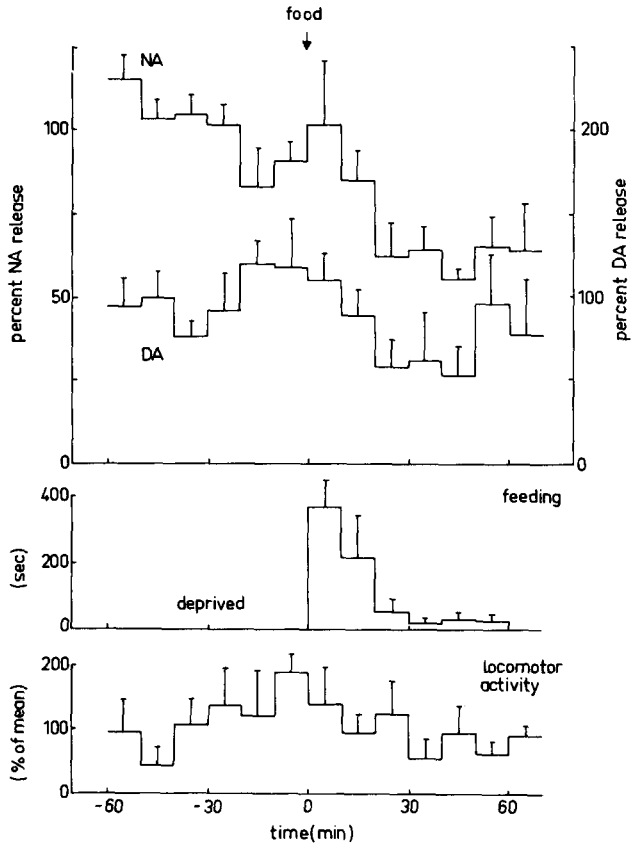

FIG. 3. NA and DA release from the medial hypothalamus of 16 hr food deprived rats which were subsequently given free access to food (indicated by arrow). Data were obtained from 4 animals. Catecholamine release is expressed as percentage of the mean value before food presentation in the respective experiment. Duration of feeding and percent locomotor activity over 10-min periods are also indicated. For each value the SEM is depicted.

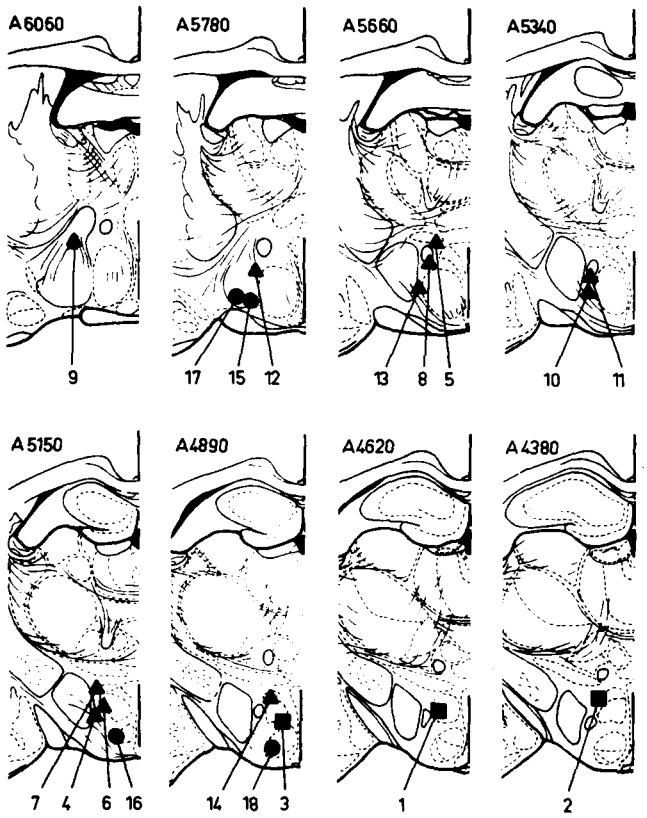

FIG. 4. Diagrammatic representation of hypothalamic perfusion sites. The loci are projected on frontal sections according to the atlas of König and Klippel [12]. When two overlapping sites were perfused, only one is plotted. The increase in hourly food intake induced by local application of L-NA (20 nmoles) is indicated for each site: •≤1.2 g; 1.2<▲≤2.4 g; ■>2.4 g. Inferior numbers represent the rank order in terms of the magnitude of the NA effect.

where $\Sigma = \sum_{i=1}^{n}$; x_i, ith value of the x series; y_{i+k}, (i+k)th value of the y series; n, number of values per series minus k. In the test of serial correlation the x and y series are identical. SCCs were computed at time lags 1 to 13. Since the shortest series (3 h) consisted of 18 values, at least 5 pairs of values (n) were used for the calculation of the SCCs. The statistical significance of a SCC was determined on the basis of the *t*-value expressed by the equation

$$t = r_k \sqrt{\frac{n - 2}{1 - r_k^2}}$$

When no significant SCCs were found, the time series was considered to be stationary.

The relation between CA release and the occurrence of a particular behavior was computed from stationary release patterns. CA release data were expressed as percentages of the mean release in each respective experiment. Means of NA and DA release were then calculated for 10-min sampling periods which included the particular behavior, as well as for the preceding and following 10-min periods. Behavioral activity was only considered when it lasted for at least 30 sec during a 10-min period. Further statistical evaluation of CA release values was based on Student's *t*-test.

The correlation between NA or DA release and locomotor activity was determined by cross correlation analysis. Locomotor activity scores expressed as percentage of mean were designated as the x series and percent CA release data as the y series. Correlation coefficients (r_k) at the time lags − 2 to 3 were calculated. For the actual computation of r_k at a negative time lag, the x and y series were transposed. The calculation of a cross correlation coefficient for several pairs of series was essentially identical.

RESULTS

Spontaneous Release and Drug Effects

Release of NA was observed at each of 17 sites in the hypothalamus of untreated rats which had free access to food. Mean NA release over a period of several hours ranged from 2.5 to 18.8 pg/min. DA release was observed at the majority of the sites; the highest mean value measured was 3.4 pg/min. Adrenaline was not detected in the perfusates.

The effects of drugs which probably act on adrenergic neurotransmission are illustrated in Fig. 2. A gradual increase in hypothalamic NA release was observed up to 75 min after the administration of reserpine in combination

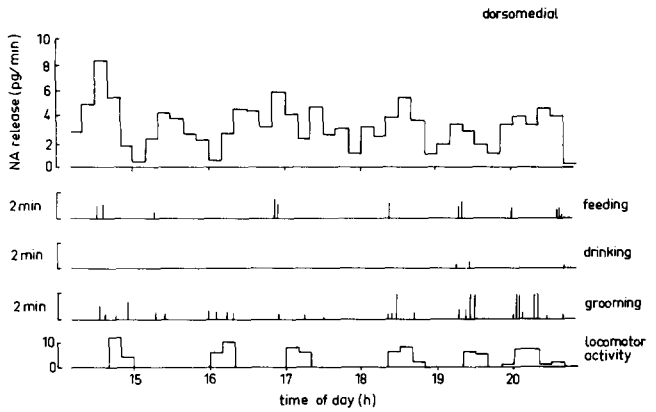

FIG. 5. Representative recording of a perfusion experiment with a rat which had a cannula in the dorsomedial hypothalamus (site 14, Fig. 4). CA release over 10-min periods is depicted. The onset time and duration of feeding, drinking and grooming, as well as the locomotor activity scores are also illustrated.

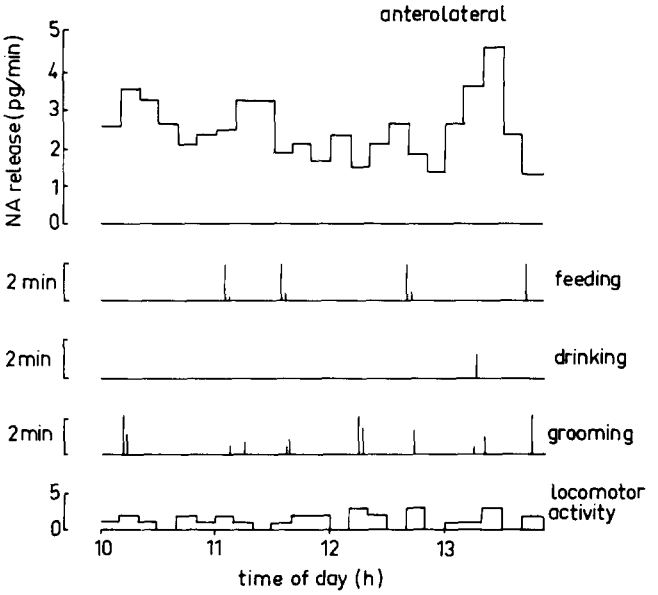

FIG. 7. Representative recording of a perfusion experiment with a rat which had a cannula in the anterolateral hypothalamus (site 15, Fig. 4). See also Fig. 5.

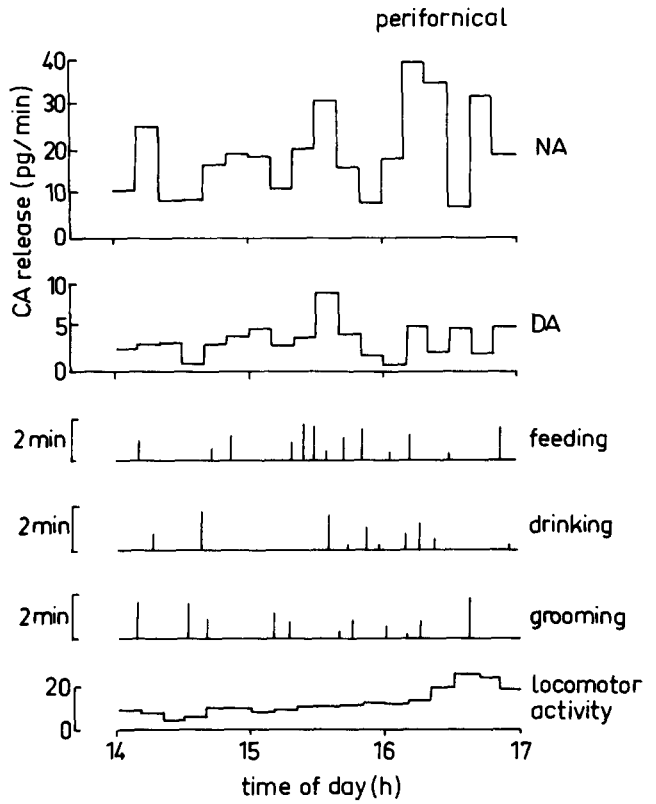

FIG. 6. Representative recording of a perfusion experiment with a rat which had a cannula in the perifornical hypothalamus (site 11, Fig. 4). See also Fig. 5.

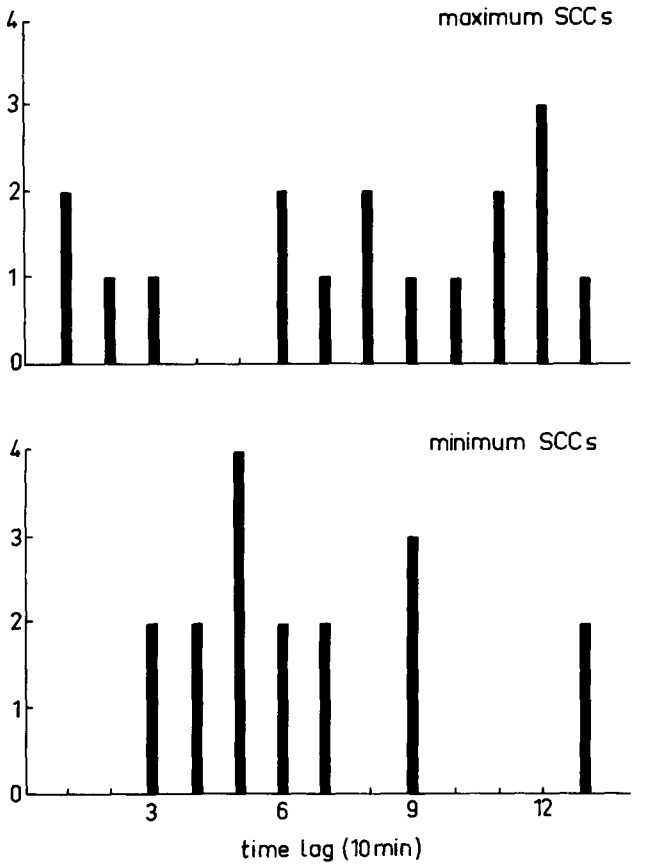

FIG. 8. Histograms of time lags corresponding to the maximum (upper panel) and minimum (lower panel) serial correlation coefficients (SCCs) for 17 NA release patterns. Catecholamine release over 10-min periods was measured in the hypothalamus of rats which had free access to food.

with desmethylimipramine. In addition, a stimulation of NA release was found after two different doses of D-amphetamine in urethane anesthetized rats.

Food Deprivation

CA release from the medial hypothalamus was measured in 4 rats which had been deprived of food for 16 hr and were subsequently given free access to food. Chemical and behavioral data are depicted in Fig. 3. Food intake was high during the 20 min immediately after food presentaion whereas the animals appeared to be satiated during the next 50 min. The locomotor activity patterns of the 4 experiments were found to be stationary. In contrast, the time series consisting of NA release data were not. Before food presentation, mean NA release over 10-min periods was consistently higher than during satiety. A similar phenomenon was not observed for DA release from the same sites; in fact, the DA release patterns were stationary. However, a significant positive correlation between DA release and locomotor activity was found at a time lag of 10 min ($r = .350$, $p<0.05$).

Free Feeding

CA release from various sites in the hypothalamus was determined in rats which had free access to food. The perfusion sites are depicted in Fig. 4. The sensitivity to exogenous NA in respect of food intake stimulation is indicated. A total of 14 sites out of 18 were considered to be sensitive since a mean increase in hourly food intake of more than 1.2 g [2,6] was observed after local application of 20 nmoles of L–NA. Numbers represent the rank order in terms of the magnitude of the NA effect.

Recordings of representative perfusion experiments are given in Figs. 5, 6 and 7. They clearly demonstrate that local CA release measured over 10-min periods varies over time. In order to investigate whether a discrete general periodicity exists, time series analyses of NA release data obtained in 17 experiments with rats which had ad lib access to food were compared. All patterns were stationary. Time lags corresponding to the maximum and minimum SCCs were determined for each of the time series and represented in histograms (Fig. 8). Although the minimum SCCs occurred predominantly at lags 3 to 7, neither maximum nor minimum SCCs were found to produce discrete peaks.

Mean NA and DA release over 10-min periods before, during and after the occurrence of feeding, drinking or grooming were calculated for the dorsomedial, perifornical, subfornical, and anterolateral areas of the hypothalamus. The calculations were based on the stationary CA release patterns obtained in 11 perfusion experiments. CA release during a particular behavior, as well as release at 10 min after that, was respectively compared with CA release at 10 and 20 min before, and 20 and 30 min after the behavior by means of *t*-tests. The results of the calculations and statistical analyses are summarized in Fig. 9.

Significant increases (40–50%) in NA release from the dorsomedial and perifornical areas were observed during a 10-min period which included feeding (Fig. 9a). It should also be noted that NA release from these areas during feeding was higher than the mean release (100%) during the whole perfusion experiments. NA release from the subfornical area was found to be increased at 10 min after the act of feeding whereas no significant changes in NA release from the anterolateral hypothalamus were observed around the time of feeding. The mean increase in hourly food intake induced by locally applied L–NA (20 nmoles) was as follows: dorsomedial, 2.2 g; perifornical, 1.7 g; subfornical, 1.5 g; anterolateral, 0.6 g (see also Fig. 4).

DA release from the dorsomedial, perifornical and subfornical areas, respectively, was not significantly changed during feeding or immediately afterwards (Fig. 9b). (Release of DA was not detected at the two sites in the anterolateral hypothalamus.) In none of the areas investigated were significant changes in NA or DA release found around the incident of drinking or around the occurrence of grooming. Moreover, NA release was not altered during 10-min periods which included drinking and/or grooming (Fig. 9c).

Tests of cross correlation performed on the data from the same sets of experiments revealed no significant correlations between DA release and locomotor activity. However, a significant positive correlation ($r = .369$, $p<0.025$) between NA release from the anterolateral hypothalamus and locomotor activity was observed at a time lag of 10 min (Fig. 9d). A significant correlation between NA release from the dorsomedial area and locomotor activity at a time lag of −10 min was also found although this correlation was obviously artefactual since in the 3 experiments concerned, a highly significant correlation ($r = .545$, $p<0.001$) between duration of feeding and locomotor activity existed at the same time lag. No significant correlation coefficients were found when NA release from the perifornical or the subfornical area was compared with locomotor activity.

Angiotensin–Induced Drinking

Further investigations of the specificity of changes in hypothalamic NA release were based on the relation between angiotensin-induced drinking and NA release. Perfusions were performed in the perifornical area of rats which had ad lib access to food and water. The data obtained in 4 different perfusion experiments are presented in Fig. 10. Angiotensin consistently elicited a drinking response of about 3.5 ml within 10 min after intraventricular administration whereas food intake was constantly low. No effect on locomotor activity was observed. The NA patterns of the different experiments were all stationary. During the 10-min period after the injection, NA release from the perifornical hypothalamus was not significantly changed when compared with release during each of the pre- and postdrinking intervals.

DISCUSSION

This study provides direct evidence of the spontaneous release of endogenous NA and DA from the rat hypothalamus in vivo. The results show that alterations in local CA release measured over 10-min periods normally occur. It appears that NA release from the medial hypothalamus depends on experimental feeding conditions. From studies in freely feeding rats it is concluded that particularly NA release from the dorsomedial and perifornical areas of the hypothalamus is elevated during feeding but not during drinking or grooming.

The concentrations of endogenous CA in the perfusate most probably reflect the functional release from nerve terminals. This is substantiated by the observation that drugs which are assumed to increase NA concentration in

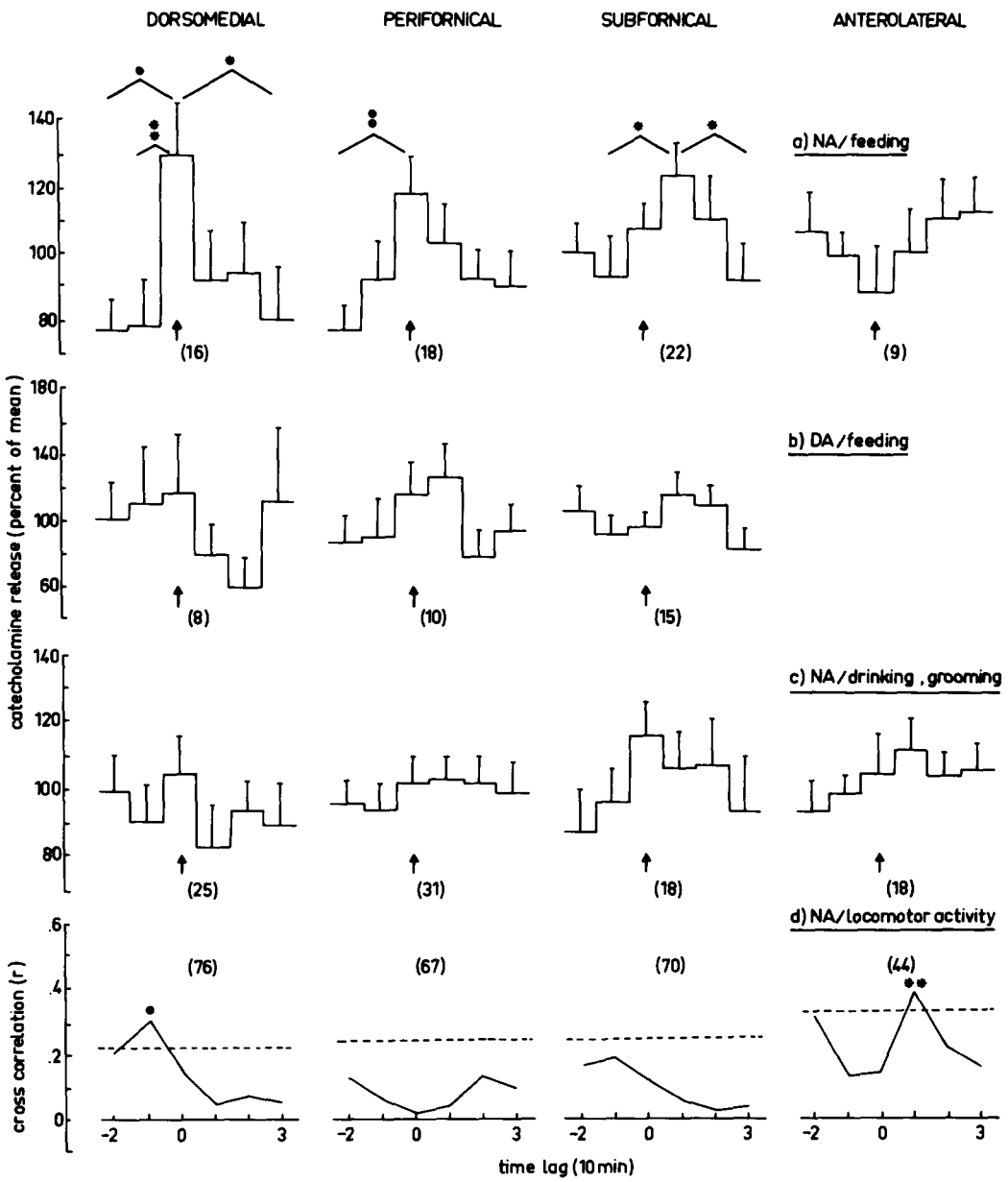

FIG. 9. Relation between CA release from different hypothalamic areas and behaviors observed in rats which had free access to food. Calculations were based on release patterns obtained in 11 experiments. The perfusion sites are indicated in Fig. 4; dorsomedial, sites 2, 3 and 14; perifornical, sites 7 and 11; subfornical, sites 10 and 13; anterolateral, sites 15 and 17. CA release over 10-min periods is expressed as percentage of the mean in the respective experiment. For each value the SEM is depicted. A 10-min period which included a particular behavior is indicated by an arrow; the total number of such periods is represented within parentheses. A behavior was only considered when it lasted for at least 30 sec during a 10-min period. (a) NA and (b) DA release over 10-min periods before, during and after the occurrence of feeding; (c) NA release over 10-min periods before, during and after the occurrence of drinking and/or grooming. (d) Cross correlation between NA release and locomotor activity (total number of values for each parameter is represented in parentheses). Statistical significance of the difference between means or a coefficient of cross correlation is indicated: $*p<0.05$, $**p<0.025$, $\bullet p<0.01$, $\bullet\bullet p<0.005$.

the synaptic cleft [4,9] cause elevations in the amount of NA in the perfusate (Fig. 2). This finding also indicates that stimulation of neuronal CA release is not entirely masked by physiological processes of extraneuronal degradation and reuptake into nerve terminals, although the amounts of CA found in the perfusate do not necessarily equate the amounts released at the synaptic junction. Furthermore, variations in NA release over time are evident under normal conditions, whereas it has been found that these do not reflect a general autonomic rhythm with a fixed period of 2 hr or less (Fig. 8). Finally, CA in hypothalamic perfusates do not arise from blood since the mean NA concentrations

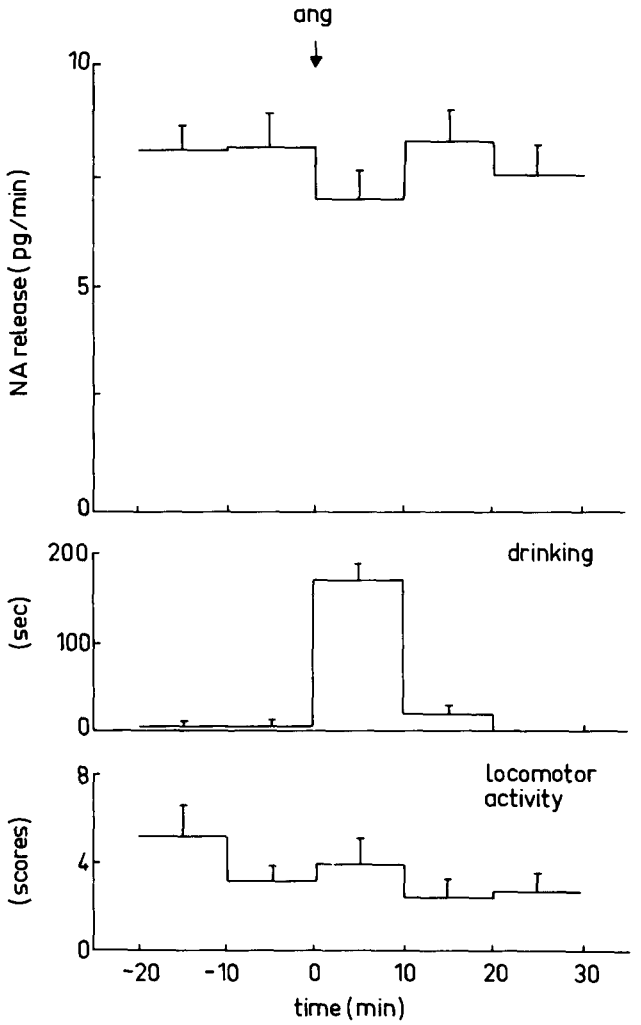

FIG. 10. The relation between NA release from the perifornical hypothalamus and angiotensin-induced drinking. The perfusion sites are indicated in Fig. 4 as sites 7, 8 and 12. Data were obtained from 4 experiments; a total of 10 angiotensin injections (ang, indicated by arrow) was given at intervals of at least 60 min. The SEM is indicated.

measured are generally even higher than the NA level of 0.4 ng/ml found for rat plasma obtained from an iliac cannula (unpublished).

NA release from the medial hypothalamus of 16 hr food deprived rats is shown to be diminished after the animals have been allowed to satiate themselves (Fig. 3). This decrease can not be attributed to a change in locomotor activity. Obviously, a similar phenomenon was not detected in the efflux of radioactivity after the injection of labeled NA [15] since this parameter normally decreases over time. The relatively high NA release during starvation also explains the enhancement of α-methyl-*p*-tyrosine induced NA depletion in the hypothalamus of 22 hr food deprived rats [8]. It might reflect increased activity of hypothesized NA neurons which inhibit a hypothalamic satiety mechanism [13] in hungry rats. The significant correlation between DA release and locomotor activity might indicate that the changes in hypothalamus DA metabolism observed in food deprived rats [8] were related to alterations in locomotor activity which may occur during food deprivation [20].

The data on NA release during 10-min periods before, during and after feeding in rats which had free access to food (Fig. 9a) indicate that an anatomical differentiation exists in the functional activity of hypothalamic NA neurons. Substantial elevations were found in the dorsomedial and perifornical areas during feeding. Such an enhancement in NA release was not found in the anterolateral hypothalamus whereas in the subfornical area a peak in NA release occurred at about 10 min after the act of feeding. This delay in NA release may reflect diffusion from a site of actual release. The results suggest that the activity of NA neurons which project into the dorsomedial and perifornical areas is specifically related to feeding behavior. The data corroborate the regional differentiation of the effect of exogenous NA on food intake; the highest sensitivity was observed in those areas (Fig. 4). Our findings are in accordance with the results of previous localisation studies which pointed to a region which includes the dorsomedial [10] and rostral perifornical areas [6, 14, 24] as being very sensitive to NA's action. The increased NA release from these areas during feeding corresponds well with the anatomically specific increases in NA level and turnover observed in the dorsomedial nucleus and the rostral hypothalamic region dorsal to the fornix in rats showing relatively high feeding rate [25].

The data on DA release from the dorsomedial and perifornical hypothalamus indicate that changes in the activity of DA neurons in these areas do not occur during feeding (Fig. 9b). This is in agreement with the earlier finding that DA metabolism in the dorsomedial nucleus and the perifornical region is not changed in rats showing high feeding rate when compared to values for rats showing low feeding rate [25]. The conclusion that DA neurons in these regions are not directly involved in feeding behavior is also substantiated by the observation that local application of DA does not immediately elicit feeding [3,24].

The activity of NA neurons which terminate in the dorsomedial and perifornical areas of the hypothalamus appears to be specifically related to feeding. No changes in NA release during or immediately after drinking and/or grooming were observed in any of the four areas investigated (Fig. 9c). Moreover, no significant cross correlation was found between NA release from the perifornical or the subfornical area and locomotor activity (Fig. 9d) whereas the significant correlation coefficient observed for the dorsomedial areas has been shown to be an artefact. The correlation between NA release from the anterolateral hypothalamus and locomotor activity explains the increase in NA metabolism in the rostral medial forebrain bundle during the night compared to daytime values [25]. This increase was supposed to be not specifically related to alterations in feeding behavior which is also substantiated by the NA release data. However, it is not clear whether the medial forebrain bundle NA neurons concerned underlie locomotor activity per se; they may also be involved in general arousal or sleep-wakefulness patterns [5, 11, 21].

Drinking is, at least in part, closely associated with meals. It has also been demonstrated that local application of L-NA in especially the perifornical area elicits a drinking response as well as feeding [14,24]. However, even during the consistently vigorous drinking response resulting from the intraventricular injection of angiotensin [7], no significant change in NA release from the perifornical

hypothalamus was observed (Fig. 10). This is additional evidence that NA neurons in the direct vicinity of the fornix columns are not directly involved in the act of drinking but specifically underlie feeding behavior.

The results of this study confirm the concept that NA containing neurons associated with the dorsomedial nucleus and the rostral perifornical region are implicated in the act of feeding [25]. It is not clear whether the same populations of neurons are also responsible for the observed enhancement of NA release upon food deprivation. The elevation of NA release particularly during feeding is in accordance with the obervation that low doses of exogenous NA infused into the forebrain of rats are only effective during a meal [23]. Although no data on endogenous adrenaline release were obtained as yet, adrenaline neurons in the dorsomedial nucleus might also be implicated in feeding behavior, as suggested earlier [25].

The procedure used represents a chemical method for the continuous registration of neuronal activity in small areas of the brain in unanesthetized rats. Therefore, it is considered to be an alternative of electrophysiological recording techniques [5,18] in various types of experiments, whereas it enables the measurement of activity of particular types of neurons. The detection of changes in NA release during feeding demonstrates that the method may be a useful tool in the identification of physiological mechanisms in the central nervous system.

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