with local angiotensin in the brain or other tissue is therefore of practical importance.

These data also have theoretical implications. ANG II is present in the blood, kidney (*17–19*), adrenal gland (*19, 20*), and brain. This adds evidence to the theory that the same substance may act as a circulating blood hormone, tissue factor, neurohormone, or neurotransmitter (*21*). The occurrence of angiotensin in the central nervous system, endocrine glands, and blood may thus explain its coordinating function in cardiovascular regulation, which includes direct control of peripheral resistance and blood volume as well as complex behavior such as thirst and salt appetite.

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22. The angiotensin peptides were purchased from Beckman, Palo Alto, Calif., and Bachem, Bubendorf, Switzerland. This work was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 90, Cardiovasculäres System. The secretarial help of I. Büchler and technical assistance of F. Bles and Th. Fleck is acknowledged.

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Morphine Tolerance in Genetically Selected Rats Induced by Chronically Elevated Saccharine Intake

Abstract. *Rats of line LC2-Hi that drank about 50 milliliters of a highly palatable saccharine solution daily for 28 consecutive days did not show morphine analgesia or an opioid form of stress-induced analgesia and were not responsive to naloxone. These findings support the idea that chronically elevated saccharine intake may cause increased release and utilization of endogenous opiates.*

There is evidence that the ingestion of sweet substances is, in part, under the control of endogenous opioids. Specifically, naloxone, an opioid antagonist, markedly reduces the ingestion of a sweet solution (*1, 2*) while having only moderate effects on drinking in response to systemic dehydration (*3*). Furthermore, the opioid agonist morphine causes a threefold increase in consumption of a 0.5 percent saccharine solution (*4*) while exerting little effect on water intake after fluid deprivation (*2*). Thus activation of the opioid system enhances the intake of sweet solutions. It has not been known whether the reverse relation also holds, that is, whether increased ingestion of a saccharine solution results in a greater release and utilization of endogenous opioids. If such a relation exists, one would expect animals consuming large volumes of saccharine solution over long periods to be more tolerant to morphine than their controls. We now report that repeated consumption of a 3 m*M* sodium saccharin solution induced cross-tolerance to morphine and to stress-induced analgesia.

The availability of LC2 lines of rats made this experiment feasible (*5*). The LC2-Hi line (*6*), especially the females, are distinguished by elevated rates of

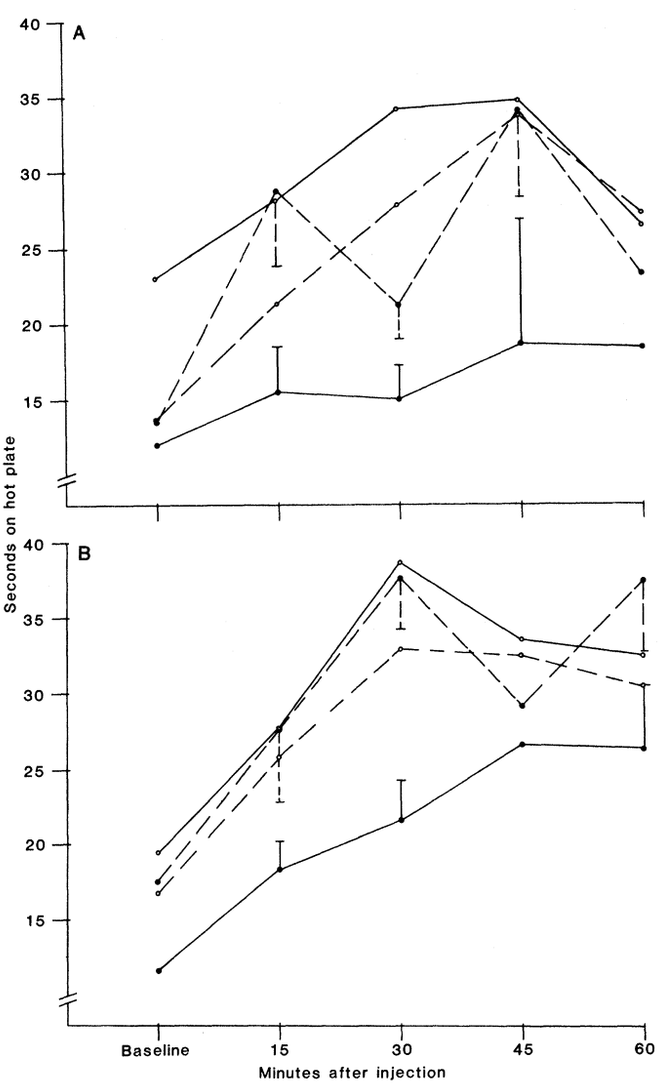

Fig. 1. Mean latencies of the pain response after the injection of morphine hydrochloride at 2.5 mg/kg (A) and 5.0 mg/kg (B). Open and closed circles represent LC2-Lo and LC2-Hi lines, respectively. Dashed and continuous lines represent water and saccharine exposure, respectively. Vertical lines represent standard errors of the means of LC2-Hi groups consuming water or saccharine.

intracranial self-stimulation and excessive drinking of saccharine. In contrast, LC2-Lo rats exhibit relatively low rates of self-stimulation and consume relatively little saccharine (*7*). Ten females of each line were given unlimited 3 m*M* sodium saccharin in deionized water for 28 consecutive days, while six LC2-Hi and seven LC2-Lo females were given only deionized water during the same period. All rats were individually housed and received standard laboratory rat food.

After the 28-day period of fluid exposure, each rat was tested on a hot plate in three sessions (*8*). The sessions were separated by 2 days, during which the assigned fluids remained available. Each session consisted of five exposures to the hot plate. The first exposure in a session preceded any drug injection. Then each animal received the appropriate drug and was retested 15, 30, 45, and 60 mintues later. In the first session half of the rats in each group received a subcutaneous injection of morphine hydrochloride (2.5 mg/kg; Endo) dissolved in isotonic saline, while the remaining animals received saline only. In the second session all rats received a subcutaneous injection of morphine hydrochloride (5.0 mg/kg). In the third session rats that had previously received morphine at 2.5 mg/kg were injected with isotonic saline, while the remaining animals were given a subcutaneous shot of naloxone hydrochloride (5.0 mg/kg; Endo). Testing and scoring were done without knowledge of the solution drunk, genetic line, and drug injected, except in the second session, when all the rats were treated identically. The dependent variable was latency of the pain response (withdrawal and licking of a rear paw) to the hot plate (*9*).

Water intake, food consumption, and body weight did not differ significantly between the two genetic lines (*10*). However, the LC2-Hi group ingested a mean of 50 ml of saccharine solution daily, while the LC2-Lo group drank only about 24 ml daily [$F(1, 29) = 16.07$, $P < 0.05$].

Rats of the LC2-Hi line drinking saccharine (group HS) did not increase their response latency after receiving morphine at 2.5 mg/kg (Fig. 1A). In contrast, LC2-Lo rats ingesting saccharin (group LS) and LC2-Hi and LC2-Lo rats drinking water (groups HW and LW, respectively) all increased the latency of their pain response. Morphine at 5.0 mg/kg did increase the latency of the pain response for the HS group, but the change was not as profound as that for the other three groups (Fig. 1B). Analysis of variance revealed a significant interaction between genetic line and solution drunk as observed at the peak of morphine analgesia [$F(1, 29) = 8.25$, $P < 0.05$].

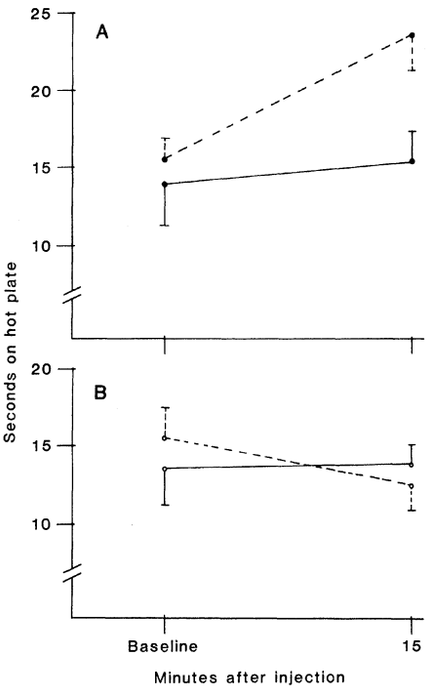

Fig. 2. Mean latencies of the pain response before and 15 minutes after the injection of saline (A) and naloxone hydrochloride (B). Dashed lines represent groups LW, HW, and LS and continuous lines represent group HS.

On the basis of these findings, stress-induced analgesia should not have appeared in the HS group, which exhibited morphine tolerance. Moreover, naloxone should not have affected the latency of the pain response in HS rats but should have decreased the elevated response thresholds in the other three groups (*11*). Indeed, HS rats did not exhibit stress-induced analgesia. Their response latency in the second hot plate trial, unlike that of the other groups, did not differ from that in the first trial (Fig. 2A). Naloxone eliminated stress-induced analgesia in the HS rats but did not affect their response latency in the second hot plate test (Fig. 2B). The absence of stress-induced analgesia in these rats makes it unlikely that the relative ineffectiveness of morphine and naloxone reflects inefficient absorption of the drugs.

These findings strongly suggest that chronically elevated intake of a highly palatable saccharine solution causes changes in rat behavior that are consistent with elevated opiate levels and tolerance to opioids. These findings cannot be explained on the basis of a genetic bias alone, since LC2-Hi rats drinking water only showed the same morphine analgesia, stress-induced analgesia, and blockade of the latter by naloxone as did LC2-Lo animals. The critical factor is the elevated consumption of a sweet solution (the relative importance of volume intake, excessive saccharine intake, or attaining a certain level of saccharine in the plasma on a long-term basis remains unknown). The fact that only the LC2-Hi line demonstrated the phenomenon is of considerable interest. These rats may serve as a valuable model for addictive behavior, especially when contrasted with their natural controls, LC2-Lo rats.

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5. The LC2-Hi and LC2-Lo rat lines are selected from a common parent population for their high and low rates of lateral hypothalamic self-stimulation, respectively (*6*). The rats in this experiment belong to the ninth selection generation. The mean rates and standard errors of lateral hypothalamic self-stimulation for this generation were 2157 ± 301 and 1170 ± 210 bar presses per hour for LC2-Hi and LC2-Lo rats, respectively.
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7. The following traits are genetically related to the readiness to self-stimulate in the LC2 population. LC2-Hi rats consume more sodium saccharin than LC2-Lo rats over a wide concentration range [J. Ganchrow, I. Lieblich, E. Cohen, *Physiol. Behav.* **27**, 971 (1981); E. Cohen, I. Lieblich, J. Ganchrow, *Behav. Neural Biol.*, in press]. After the septal and ventromedial hypothalamic systems are damaged, LC2-Hi rats exhibit the expected increase in irritability while LC2-Lo animals show a diminished response [I. Lieblich, E. Cohen, B. Z. Marom, J. Dymshitz, *Brain Res.* **185**, 253 (1980)]. LC2-Hi rats eat more than LC2-Lo rats on stimulation of the lateral hypothalamus and eat less in response to stress [E. S. Valenstein, I. Lieblich, R. Dinar, E. Cohen, S. Bachus, *Behav. Neural Biol.* **34**, 271 (1982)]. Finally, LC2-Hi rats tend to react more by self-mutilation (as induced by peripheral nerve injury) than LC2-Lo rats [R. Inbal, M. Devor, O. Tuchendler, I. Lieblich, *Pain* **9**, 327 (1980)].

8. Animals were maintained on a 12:12 hour light-dark cycle (lights on at 0730) at about 22°C. Experimentation began at 0830. Animals were tested consecutively in batches of seven scrambled with respect to solution drunk and genetic background so that at least one representative of each group was included in each batch.
9. Animals were placed for 1 minute into a 20 by 20 by 40 cm plexiglass chamber whose floor was a copper hot plate covered by cardboard insulation. After this acclimation period the insulation was withdrawn and time was measured by stopwatch until the animal licked one of its hind paws or 45 seconds elapsed. The animal was then returned to its cage. Two observers were used to determine occurrence of the pain response. The hot plate was maintained at 53°C by a Fried Electric heater and thermostat, which circulated heated water through uniformly distributed tubing beneath the copper floor. Temperature was also monitored independently of the thermostat by a thermometer, the tip of which was submerged in the heated water.
10. Mean daily consumption of water was 21 and 25 ml and mean initial body weight was 263 and 255 g for LC2-Lo and LC2-Hi rats, respectively. No significant differences in gains in body weight between the two lines and among the different drinking groups were detected during the study. The mean amount of food consumed in 72 hours was 50 and 52 g for LC2-Lo and LC2-Hi rats, respectively. No significant differences in the quantity of food consumed in a second food consumption measurement were detected between lines or drinking groups.
11. The opioid form of stress-induced analgesia is partly defined by its sensitivity to blockade by naloxone [J. W. Lewis, J. T. Cannon, J. C. Liebeskind, *Science* **208**, 623 (1981)].
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Measurement of Suppressor Transfer RNA Activity

Abstract. *Transfer RNA (tRNA) suppression of nonsense mutations in prokaryotic systems has been widely used to study the structure and function of different prokaryotic genes. Through genetic engineering techniques, it is now possible to introduce suppressor (Su^+) tRNA molecules into mammalian cells. A quantitative assay of the suppressor tRNA activity in these mammalian cells is described; it is based on the amount of tRNA-mediated readthrough of a terminating codon in the influenza virus NS1 gene after the cells are infected with virus. Suppressor activity in L cells continuously expressing Su^+ ($tRNA^{tyr}$) was 3.5 percent and that in CV-1 cells infected with an $SV40^-$ Su^+ ($tRNA^{tyr}$) recombinant was 22.5 percent.*

The availability of SV40 vectors containing amber suppressor (Su^+) $tRNA^{tyr}$ (tyrosine transfer RNA) genes (*1*) and the establishment of eukaryotic cell lines (*1*) expressing functional suppressor tRNA's give promise of a new approach to the genetic analysis of cellular and viral genomes. Development of this new technology requires a rapid and quantitative assay for the level of suppressor activity in a particular system. The genetic structure of influenza virus provides an ideal system to measure the suppressor tRNA mediated readthrough of stop (terminating) codons.

Extensive studies on the structure of the NS gene of different influenza virus field isolates have shown that the NS1 gene products may vary in length. For example, the NS1 protein of the 1947 strain A/FM/1/47 is only 202 amino acids while the A/USSR/90/77 virus NS1 gene product contains 237 amino acids (*2*). Another field virus isolate, A/PR/8/34 virus, has a long NS1 protein (230 amino acids) (*2*). The NS genes of these three viruses all terminate the NS1 protein via a UGA (U, uracil; G, guanine; A, adenine) codon (opal codon). In contrast, A/CAM/46, another influenza virus field isolate, directs the synthesis of a 216 amino acid NS1 protein terminating with an amber codon (UAG). This amber stop signal is followed downstream by a UGA triplet at amino acid position 237 (*2*). Therefore, amber suppression is expected to produce an NS1 polypeptide with an additional 20 amino acids. Since NS1 polypeptides of length 237 are found in other field variants, it is likely that both the terminated (at position 216) and suppressed (at position 237) proteins would be stable.

Virus stocks of SV40 recombinants containing active amber Su^+ $tRNA^{tyr}$ genes have been described (*1*) and have been shown to suppress a nonsense (UAG) mutation in the thymidine kinase gene of herpes simplex virus mutants (*3*).

Table 1. Suppression of termination at NS1 amber codon. The SV40 recombinants and L cell lines used have been described (*1*). The amber readthrough was measured by infection of cells with influenza A/CAM/46 virus as described in the text. The values of the influenza virus NS1 readthrough protein represent the average of two independent experiments. Conditions for infection of CV-1 cells and for L cells were as described for Fig. 1, lanes 7 and 8.

Cell	Amount (%)
CV-1 plus Su^+ SV40 recombinant	22.5
CV-1 plus Su^- SV40 recombinant	< 0.5
L cell control	< 0.5
L cell line 37	3.6
L cell line 39	3.4

Infection of CV-1 cells with these stocks results in accumulation of the Su^+ tRNA to 2 to 5 percent of the total cellular tRNA.

To test the readthrough of the amber codon in the NS1 coding region, CV-1 cells were infected with A/CAM/46 virus in the presence of SV40 recombinants containing a Su^+ or a Su^- tRNA gene. Infection of CV-1 cells for 24 hours with the SV40 recombinants containing the Su^+ tRNA gene resulted in the production of suppressor activity to give partial readthrough of the NS1 protein (Fig. 1, lane 8, arrow). In contrast, infection with the equivalent Su^- SV40 recombinant did not permit synthesis of such a polypeptide in A/CAM/46 virus infected CV-1 cells (Fig. 1, lane 7). Similar results were obtained when CV-1 cells were infected with the SV40 recombinants for 48 hours prior to the addition of A/CAM/46 virus (Fig. 1, lanes 9 and 10). However, no suppressor tRNA activity was detectable in cells that were coinfected at the same time with the SV40 recombinants and A/CAM/46 virus (Fig. 1, lane 6). The gel migration of the additional protein band in lanes 8 and 10 of Fig. 1 is in agreement with the predicted length of the readthrough product of the NS1 gene of A/CAM/46 virus.

The identity of this readthrough product, designated by the arrow in Fig. 1, was confirmed in a subsequent experiment with a monospecific rabbit antiserum to bacterially synthesized NS1 protein (*4*). This antiserum precipitates both the NS1 polypeptide as well as the suppressed readthrough product from an infected cell extract (Fig. 2, lane 3). In contrast, when cells are coinfected with the Su^- SV40 recombinants and A/CAM/46 virus, only the nonsuppressed NS1 polypeptide is present (Fig. 2, lanes 4 and 6). Gel separation of the labeled NS1 polypeptide and its readthrough product and analysis of the gel slices revealed a 15 to 25 percent readthrough of the amber codon in the CV-1 cells infected with A/CAM/46 virus and the Su^+ SV40 recombinants. Optimal readthrough occurs if the influenza virus infection follows an 18- to 30-hour preliminary incubation with the Su^+ SV40 recombinants (data not shown). Use of a tenfold concentrated Su^+ SV40 recombinant virus stock did not increase the amount of the readthrough product. However, a fivefold dilution of the Su^+ SV40 recombinant preparation used to infect CV-1 cells did lead to a 50 percent reduction of the amber codon suppression.

Amber suppression in cell lines carry-