We were somewhat surprised at the inability of Antelman and Rowland to reduplicate our results in view of independent confirmation that naloxone reduces tail-pinch–induced eating (1). Furthermore, we have shown that (i) naloxone’s suppressive effect on tail-pinch–induced eating could not be reversed by central administration of known pharmacological agents that enhance appetite and (ii) naloxone inhibits tail-pinch–induced eating in mice (2, 2a). The discrepancy between the experiments of Antelman and Rowland and ours may result from their use of trained animals, whereas we used naïve animals. Panksepp et al. (3) have provided evidence that endogenous opioids play a role in social behavior and learning situations. The introduction of a learning paradigm may have contributed Antelman and Rowland’s results.

Apart from the direct evidence that endogenous opioids are involved in tail-pinch–induced eating, there is a large amount of indirect evidence. Neck scruff pinch (which also induces eating) produces a cataleptic state that is reversed by prior treatment with naloxone (4). We have observed catalepsy in some animals during tail pinch. This tonic immobility observed during tail pinch closely resembles morphine catalpexy (5). The tonic immobility induced by body pinch is widely reported to be mediated by opiates and serotonin (6). Ornstein (7) has demonstrated that tail pinch will suppress wet-dog shakes in rats in a manner similar to morphine. The suppressive effect of a number of peptides on stress-induced eating is partially reversed by the concomitant administration of a long-acting enkephalin analog (2, 8a). We have found that a 10-minute tail-pinch period in the presence of wood chips produces a significant reduction in immunoreactive dynorphin [a leucine enkephalin containing endogenous opiates that induce feeding (9)] levels in rat brain.

If endogenous opioids were involved in tail-pinch behaviors, one would expect tail pinch to produce analgesia. Antelman et al. noted that tail pinch induced apparent independence to pin prick (10). Tail pinch also produces naloxone reversible analgesia when the hot-plate test is used (11). Pain (nociception) is a well-recognized activator of endogenous opiates (12). Antelman and colleagues (10) have consistently argued that tail pressure when applied correctly is not painful. However, Rowland and Marques (13) point out that, at the very least, tail pinch represents an annoying stimulus and that the “demarcation between pain and annoyance (or stress) is a fuzzy line at best.” Other evidence that nociception plays an integral role in tail-pinch behaviors includes the following: (i) Tail-pinch behaviors are blocked by a local anesthetic ring block of the tail; (ii) painful stimuli applied to other parts of the body, such as foot and neck, induce feeding; and (iii) diabetic animals with increased tail-flick latencies have a prolonged latency for induction of tail-pinch behaviors compared to their littermate controls (2a, 14).

We thank Donna Kocan for help with the experiments.

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Antelman has stressed the similarities between the neural effects of amphetamine and tail pinch. Amphetamine-tolerant guinea pigs exhibit supersensitivity to naloxone with respect to feeding behavior (15). Long-term amphetamine exposure results in higher levels of β-endorphin in the hypothalamus in guinea pigs and acute infusion of dextroamphetamine induces increases in plasma β-endorphin in humans (16). These findings suggest that endogenous opiates have a role in amphetamine-induced behaviors and thus by implication in tail-pinch–induced behaviors.

Antelman and Rowland observed few withdrawal behaviors in that their total tail-pinch time amounted to less than 20 percent of ours. However, they previously reported the induction of tolerance to food ingestion in rats subjected to tail pinch over a long period and commented that the rats became “agitated” (17); both of these observations provide further evidence for a possible autoaddiction to endogenous opiates resulting from long-term tail pinch. As mentioned (7), the ability of tail pinch to block wet-dog shakes is particularly relevant in this regard.

Thus, there appears to be adequate evidence supporting the view that endogenous opiates are involved in tail-pinch–induced food ingestion. Although we (18) originally also took the view that tail pinch represents a model of stress-induced eating, our ongoing studies have led us to believe that the predominant tail-pinch behavior is gnawing (chewing), with any associated eating representing an epiphenomenon. Support for this concept comes from the observation that tail-pinch activation is mediated by dopaminergic mechanisms (10), since dopaminergic agonists are recognized as producing oral stereotypy rather than ingestion (19). In addition, Rowland and Marques (13) have stated that “because many rats shred the food without eating we believe that ingestion may be incidental to the predominant motor act of bitting.” Should this be the case, the argument presented above by Antelman and Rowland that the decreased eating in our rats was due to increased gnawing would not apply. Our response to the final paragraph of Antelman and Rowland is included in (20).

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Limitations in Identifying Neurotransmitters Within Neurons by Fluorescent Histochemistry Techniques

Grace and Bunney (1) purport positively to identify neurons as dopaminergic by intracellularly injecting L-dopa and subsequently inducing fluorescence in those cells by treating the tissue with glyoxylic acid. This method is based on the assumption that "only the dopamine reacts with formaldehyde vapor or glyoxylic acid to form fluorescent compounds" (1). On the contrary, our studies (2) show that L-dopa itself fluoresces when treated with the SPG (sucrose, phosphate buffer, and glyoxylic acid) method of de la Torre and Surgeon (3), and that the emission maximum of L-dopa is exactly the same as that of dopamine (Fig. 1). Furthermore, the products of the same biosynthetic pathway, epinephrine and norepinephrine, also fluoresce at the same wavelength when treated with glyoxylic acid. Additionally, L-dopa fluoresces if the tissue is treated by the paraformaldehyde (FMS) method (4). In this case the spectral emission of the L-dopa fluorophore is also very similar to that of the dopamine fluorophore. Therefore, it appears that neither the SPG nor the FGS methods of fluorescent histochemistry allow one unequivocally to distinguish the L-dopa fluorophore from the dopamine fluorophore.

The implication of Grace and Bunney that lack of fluorescence in the nondopaminergic cells of the zona reticulata is evidence that L-dopa is not the glyoxylic acid reactant is open to question on at least two counts. The 10 to 30 minutes allowed to elapse before the animals were killed would certainly be sufficient time for degradation of the L-dopa by either the monamine oxidase or catechol-O-methyltransferase pathways (5). There is no assurance in the report (1) that the amount of L-dopa injected into zona reticulata cells was comparable to that iontophoresed into the zona compacta of the substantia nigra. In view of the fact that L-dopa does fluoresce, careful and exact controls must be used. One would have to examine considerably more data than were given on the controls before one could reach any conclusions concerning the ability to identify dopaminergic neurons after intracellular injection of L-dopa.

The identification of putative transmitters on the basis of their fluorescence has been a problem for some time (6). When treated by any number of methods, monoamines, as well as many amino acids, fluoresce. Even tyrosine, the precursor of monoamines, has a fluorescent spectrum that would be difficult to

References and Notes

Fig. 1. Fluorescence emission spectra of L-dopa, dopamine, tyrosine, and norepinephrine in dried albumin droplets on glass microscope slides. Epi-fluorescent illumination was monitored and recorded as described (2). These are uncorrected records that are representative of at least three similar samples. The albumin was the only record which we had to set the microspectrophotometer calibration at high gain. Hence, the relative intensities of the samples and the albumin are not accurately represented. The fluorescence of the monoamines is at least 1000 times that of albumin. Readings for each sample were taken every 0.44 nm and the point plotted is an average of ten readings taken at each 0.44-nm interval.

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