

the females is associated with two stimuli. Access to and copulation with the females was accompanied by becoming the alpha, or dominant, male in a new group. It is possible that the latter experience is a provocative stimulus to increased testosterone secretion, along with frequent sexual activity. Studies are needed to clarify this issue.

With more indirect techniques of assessing testosterone secretion, such as measurement of urinary metabolites, it has been reported that testosterone secretion appeared to fall following exposure to stressful situations (6). In the rat, plasma testosterone has been observed to fall following ether anesthesia or foot shock (7). In humans, plasma testosterone has been reported to fall following surgery (8), and recently testosterone levels were observed to be suppressed in young men during the early stressful phase of officer candidate training in the army (9).

The fall in testosterone levels in the four males following defeat could be secondary to the wounding they received, as well as related to the psychological effects of such an experience. While all four males showed marked decreases in plasma testosterone, only two animals were seriously wounded. However, it is still possible that physical injury could account for the fall in testosterone levels. Preliminary results of studies in progress indicate that testosterone also falls after males are subjected to defeat and fall in dominance rank without the occurrence of physical injury.

The males in the present study were placed back in individual cages after their defeat. When defeated animals remain in the group, they assume a very depressed dominance rank and restrict their social interaction with other animals for a prolonged period of time. These observations suggest that defeat and loss of dominance is a very significant and meaningful experience for the male rhesus, and may thus present the most relevant explanation for the fall in plasma testosterone observed.

These data support the interpretation that testosterone secretion can be influenced by social and environmental variables and is not fixed. It is also possible, however, that although the pituitary-gonadal system is subject to influence by environmental events, the subsequent alterations in plasma testosterone may significantly affect behavior. It could be argued that the fall in testosterone following defeat and loss of dominance rank functions as an

adaptive response. As aggressive threats or challenges by a subordinate animal are severely punished by the dominant members of a group, high levels of testosterone stimulating such behavior could be viewed as inappropriate and maladaptive. In a parallel vein, increase in testosterone stimulated by access to females would function to support the increased frequency of sexual activity. Work is needed to clarify these issues.

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#### References and Notes

1. R. M. Rose, J. W. Holaday, I. S. Bernstein, *Nature* **231**, 366 (1971).
2. D. Mayes and C. A. Nugent, *J. Clin. Endocrinol.* **28**, 1169 (1968). Unlike the original method of Mayes and Nugent, the analyses of plasma testosterone reported here utilized only one thin-layer chromatography following extraction prior to competitive protein binding, similar to that employed by other workers, for example, J. A. Demetriou and F. G. Austin [*Clin. Chem.* **16**, 111 (1970)] and J. S. D. Winter and D. R. Grant [*Anal. Biochem.* **40**, 440 (1971)]. Recent studies in our laboratory of the specificity of this modified technique indicate it overestimates the concentration of plasma testosterone in the male rhesus by approximately 15 percent. However, this enhancement occurred at both baseline and elevated levels of plasma testosterone, and therefore does not invalidate the observation of increased levels following social and sexual stimulation.
3. J. S. D. Winter, S. Taraska, C. Faiman, *J. Clin. Endocrinol.* **34**, 348 (1972).
4. See reviews by J. M. Davidson and S. Levine, *Ann. Res. Physiol.* **34**, 375 (1972) and W. G. Luttge, *Arch. Sex. Behav.* **1**, 61 (1971). For the rabbit: M. Saginor and R. Horton, *Endocrinology* **82**, 627 (1968) and G. C. Haltmeyer and K. B. Eik-Nes, *J. Reprod. Fert.* **19**, 273 (1969). For the bull: C. B. Katongole, F. Naftolin, R. V. Short, *J. Endocrinol.* **50**, 457 (1971). For the elephant: M. R. Jainudeen, C. B. Katongole, R. V. Short, *J. Reprod. Fert.* **29**, 99 (1972).
5. A. A. A. Ismail and R. A. Harkness, *Acta Endocrinol.* **57**, 469 (1967); Anonymous, *Nature* **226**, 869 (1970) (beard growth).
6. See review: R. M. Rose, *Psychosom. Med.* **31**, 405 (1969).
7. C. W. Bardin and R. E. Peterson, *Endocrinology* **80**, 38 (1967); B. L. Fariss, T. J. Hurley, S. Hane, P. H. Forsham, *ibid.* **84**, 940 (1969); E. L. Bliss, A. Frischat, L. Samuels, *Life Sci.* **11** (part 1), 231 (1972) (foot shock).
8. K. Matsumoto, K. Takeyasu, S. Mitutani, Y. Hamanaka, T. Vozumi, *Acta Endocrinol.* **65**, 11 (1970).
9. L. E. Kreuz, R. M. Rose, J. R. Jennings, *Arch. Gen. Psychiat.* **26**, 479 (1972).
10. Supported in part by PHS grant MH 20483-01.

17 July 1972

## Narcotic Tolerance and Dependence and Serotonin Turnover

In a recent study of the effect of protein synthesis inhibitors on morphine tolerance, Loh *et al.* (1) reported that cerebral macromolecules play an important role in the development of narcotic tolerance and dependence. In a logical extension of this work involving a consideration of those reactions or enzymes associated with the putative neurohormones, Way and his co-workers (2) suggested that one of the proteins may be associated with serotonin (5-hydroxytryptamine) synthesis. This suggestion was based on the observation that serotonin turnover in the brain increases with the development of morphine tolerance. The method that Way and his co-workers used for the assessment of serotonin turnover was that of Tozer *et al.* (3), who stated that the increase of brain serotonin after monoamine oxidase inhibition could be used as a measure of serotonin turnover.

The findings of Way and his co-workers (2) have been confirmed by Maruyama *et al.* (4), who used the pargyline method of measuring serotonin turnover. However, Cheney *et al.* (5), using a "direct method" (6), have disputed the findings of Way and his co-workers and Maruyama *et al.* In our opinion, the data and calculations pre-

sented by Cheney *et al.* do not support their conclusion of a lack of relationship between morphine tolerance and brain serotonin turnover.

In their report, Cheney *et al.* used the steady-state kinetic approach and calculated the fractional rate constant ( $k_s$ ) which in a steady-state system should reflect changes in monoamine turnover. The method involves the intravenous injection of a pulse dose of [ $^3$ H]tryptophan followed by measurement of changes in the specific activity of precursor and product.

From such data Cheney *et al.* calculated  $k_s$  for the sham-operated and for the morphine-tolerant mice. However, they arbitrarily selected only one time point pair (between 30 and 50 minutes after injection) to calculate  $k_s$ . They noted that the  $k_s$  for the sham-operated mice was nearly equal to the  $k_s$  for the morphine-tolerant mice and concluded that there was no difference in serotonin turnover between these two groups. We have since calculated, from a smoothed curve of the data they reported, the  $k_s$  for all 20-minute time intervals past 50 minutes. It is clearly shown in Table 1 that for each of the five time pairs the  $k_s$  is higher for the morphine-tolerant mice

than for the control mice. Using these values of  $k_s$  to calculate the rate of synthesis of brain serotonin, one would conclude that in most time intervals mice tolerant to morphine have a higher rate of serotonin synthesis than control mice. However, we also observed that the apparent rate of serotonin synthesis declined over time, an indication that a steady state between radioactive product and precursor was probably never established. Thus, the incomplete calculations of Cheney *et al.* (5) led to an erroneous conclusion concerning the rate of serotonin synthesis.

Cheney *et al.* have also reported that the development of morphine tolerance does not affect the conversion index, which describes the *net* rate of incorporation of [ $^3$ H]tryptophan into [ $^3$ H]-serotonin at any time after the administration of the precursor. We have carried out some similar preliminary experiments with a pulse dose of [ $^3$ H]tryptophan. When [ $^3$ H]tryptophan was administered to Swiss-Webster mice (obtained from Horton Laboratories, Oakland, California), we found that the conversion index increased 50 percent in morphine-tolerant mice at 20 minutes after the administration of the precursor. We did not investigate later time intervals. At 20 minutes after [ $^3$ H]tryptophan administration the specific activity of tryptophan had increased slightly from  $3.08 \pm 0.49 \times 10^3$  disintegrations per minute per nanomole in controls to  $3.62 \pm 0.25$  in morphine-tolerant animals. However, the specific activity of serotonin had increased markedly from  $5.79 \pm 0.75 \times 10^3$  disintegrations per minute per nanomole

in controls to  $10.08 \pm 1.13$  in the morphine-tolerant animals. No change was observed in the concentrations of endogenous tryptophan, serotonin, or 5-hydroxyindole acetic acid (5-HIAA), or in the specific activity of 5-HIAA. The reasons underlying the differences between our data and those of Cheney *et al.* are unclear at present. However, it would have been helpful if Cheney *et al.* had included data on 5-HIAA, the major metabolite of serotonin.

Cheney *et al.* (5), in agreement with the results we report here, found that the specific activity of tryptophan was somewhat higher in morphine-dependent animals than in controls. They also noted a proportional increase in the specific activity of serotonin. However, they concluded that this finding "implies a more rapid transport of the amino acid from the blood into the brain, but it has no bearing on the question of serotonin turnover." This opinion is at variance with the finding that the uptake and availability of tryptophan as well as the activity of tryptophan hydroxylase regulate serotonin biosynthesis (7).

Cheney *et al.* (5) also suggested that "under some conditions the indirect pargyline method does not serve as a valid measure of serotonin turnover." It is understood that this method has an inherent disadvantage; namely, one assumes that pargyline has only one pharmacologic effect, that is, monoamine oxidase inhibition. However, Way and his co-workers (2) also estimated the rate of serotonin synthesis in morphine-tolerant mice by using probenecid to block the egress of 5-HIAA from the brain (8). Here again, the

serotonin turnover was reported to be significantly higher in morphine-tolerant mice than in the controls. Although the probenecid method, like the pargyline method, has its own limitations, both methods have been shown by Neff *et al.* (6) to yield the same results as the direct method for the turnover of brain serotonin in control animals. On the other hand, in the direct method certain difficulties arise in drug-treated animals which affect the transport of tryptophan to the brain. The calculation of  $k_s$  reflects that proportion of tryptophan that is metabolized to serotonin. However, since tryptophan hydroxylase is not a substrate-saturated enzyme, it is necessary to consider both the  $k_s$  and the specific activity of tryptophan when analyzing the rate of serotonin biosynthesis by the "direct method."

The question of whether or not brain serotonin plays a role in the mechanism of narcotic tolerance and dependence needs to be investigated still further. Various investigators (4, 5, 9) have both confirmed and refuted the original findings of Way and his co-workers (2, 10). Acceptance of a role for serotonin would hinge largely upon the demonstration that the findings in the mouse represent a phenomenon that occurs in all species capable of developing tolerance to and dependence on morphine. It may well be that the sensitivity of the serotonin system to morphine will vary from species to species. Indeed, Maruyama *et al.* (4) have noted that different strains of mice exhibit markedly different rates of serotonin turnover. Thus, depending on whether one uses the pargyline method or the direct method, it may be necessary to investigate discrete brain regions rather than the whole brain in order to highlight the effects of morphine. In this regard, Azmitia *et al.* (11) have reported an increase in tryptophan hydroxylase activity in the midbrain of chronically morphinized rats. Knapp and Mandell reported a marked elevation in tryptophan hydroxylase activity in the synaptosomes of the septal areas after the implantation of a morphine pellet in the rat (12). Furthermore, Ho *et al.* (10) have noted marked differences in serotonin turnover in various regions of the brains of morphine-tolerant mice.

For the various reasons discussed in this technical comment, the data of Cheney *et al.* do not support their conclusion, nor are they in accord with the title of their report, "Narcotic tolerance and dependence: Lack of re-

Table 1. The effect of the development of morphine tolerance on the fractional rate constant ( $k_s$ ) of serotonin biosynthesis in mice. The data were taken from Cheney *et al.* (5) with the specific activities of tryptophan and serotonin determined by the best line fit. The fractional rate constant was calculated as described by Cheney *et al.* (5); dpm, disintegrations per minute.

| Minutes after injection       | Tryptophan (dpm/nmole) | Serotonin (dpm/nmole) | Time interval (min) | $k_s$ (hr <sup>-1</sup> ) |
|-------------------------------|------------------------|-----------------------|---------------------|---------------------------|
| <i>Control mice</i>           |                        |                       |                     |                           |
| 30                            | 1300                   | 1960                  |                     |                           |
| 50                            | 620                    | 1420                  | 30-50               | 2.21                      |
| 70                            | 425                    | 1140                  | 50-70               | 1.11                      |
| 90                            | 335                    | 1000                  | 70-90               | 0.62                      |
| 110                           | 310                    | 940                   | 90-110              | .28                       |
| 130                           | 295                    | 910                   | 110-130             | .14                       |
| 150                           | 290                    | 900                   | 130-150             | .05                       |
| <i>Morphine-tolerant mice</i> |                        |                       |                     |                           |
| 30                            | 1900                   | 2660                  |                     |                           |
| 50                            | 900                    | 1980                  | 30-50               | 2.21                      |
| 70                            | 550                    | 1480                  | 50-70               | 1.49                      |
| 90                            | 425                    | 1140                  | 70-90               | 1.24                      |
| 110                           | 375                    | 970                   | 90-110              | 0.78                      |
| 130                           | 340                    | 850                   | 110-130             | .65                       |
| 150                           | 305                    | 770                   | 130-150             | .49                       |

relationship with serotonin turnover in the brain." Furthermore, one might conclude that the direct method does not serve as a valid measure of serotonin turnover under some experimental conditions.

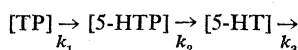
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#### References

1. H. H. Loh, F. H. Shen, E. L. Way, *Biochem. Pharmacol.* **18**, 2711 (1969).
2. E. L. Way, H. H. Loh, F. H. Shen, *Science* **162**, 1290 (1968); F. H. Shen, H. H. Loh, E. L. Way, *J. Pharmacol. Exp. Ther.* **175**, 427 (1970).
3. T. N. Tozer, N. H. Neff, B. B. Brodie, *J. Pharmacol. Exp. Ther.* **153**, 177 (1966).
4. Y. Maruyama, G. Hayashi, S. Smits, A. E. Takemori, *ibid.* **178**, 20 (1971).
5. D. L. Cheney, A. Goldstein, S. Algeri, E. Costa, *Science* **171**, 1169 (1971).
6. N. H. Neff, P. F. Spano, A. Groppetti, C. T. Wang, E. Costa, *J. Pharmacol. Exp. Ther.* **176**, 701 (1971).
7. R. J. Wurtman and J. D. Fernstrom, in *Perspectives in Neuropharmacology*, S. H. Snyder, Ed. (Oxford Univ. Press, New York, in press); A. T. B. Moir and D. Eccleston, *J. Neurochem.* **15**, 1093 (1968); J. D. Fernstrom and R. J. Wurtman, *Science* **173**, 149 (1971).
8. N. H. Neff, T. N. Tozer, B. B. Brodie, *J. Pharmacol. Exp. Ther.* **158**, 214 (1967).
9. P. J. Schechter, W. Lovenberg, A. Sjoerdsma, *Biochem. Pharmacol.* **21**, 751 (1972); I. Marshall and D. C. Grahame-Smith, *J. Pharmacol. Exp. Ther.* **179**, 634 (1971); D. R. Haubrich and D. E. Blake, *Fed. Proc.* **28**, 793 (1969); S. Algeri and E. Costa, *Biochem. Pharmacol.* **20**, 877 (1971).
10. I. K. Ho, S. E. Lu, S. Stolman, H. H. Loh, E. L. Way, *J. Pharmacol. Exp. Ther.* **182**, 155 (1972).
11. E. E. Azmitia, P. Hess, D. Reis, *Life Sci.* **9**, 633 (1970).
12. S. Knapp and A. J. Mandell, *Science* **177**, 1209 (1972).
- 10 February 1972; revised 16 June 1972

The measurement of the serotonin [5-hydroxytryptamine (5-HT)] turnover rate after pulse injection of labeled tryptophan (TP) is derived from the following model:



(where 5-HTP represents 5-hydroxytryptophan) on the assumption that  $x$  is the specific activity of TP,  $y$  is the specific activity of 5-HTP, and  $z$  is the specific activity of 5-HT.

$$\frac{dy}{dt} = k_2(x - y) \quad (1)$$

$$\frac{dz}{dt} = k_3(y - z) \quad (2)$$

The approximation of  $k_3$  ( $k_8$ ) that we reported (1) and that Hitzemann *et al.* present is derived from Eq. 3:

$$\frac{dz}{dt} = k_3(x - z) \quad (3)$$

Equation 3 is valid only for the time interval for which the value of  $x$  is very similar to the value of  $y$ . In our report (1) we assumed that Eq. 3 may be used to approximate  $k_3$  only for the time interval between 30 and 50 minutes. Moreover, we know that Eq. 3 cannot be valid throughout the entire time interval we have studied in our experiments. Indeed, the declining rate of synthesis of 5-HT calculated from our data by Hitzemann *et al.* reflects our current methodological inability to measure the 5-HT turnover rate. We have overcome this methodological barrier for measuring the turnover rate of heart norepinephrine (2), and we are currently working toward a similar method for 5-HT that will permit calculations to be made from Eq. 2.

In our report (1) we hesitated to give excessive weight to the specific activity of 5-HT of the control mice at 160 minutes because it did not appear to decline in this particular experiment (although it has in other experiments). Therefore, any calculations based on this particular point must be questionable. We have since repeated this experiment, using highly purified [ $^{14}$ C]TP. The conversion index (3) calculated for tolerant-dependent mice did not differ from that for control mice.

The probenecid and pargyline methods were excellent prototypic techniques for measurement of the rate of turnover of brain 5-HT. However, these methods do not necessarily represent ideal methods for drug studies, because they are characterized by inherent inconsistencies: pargyline interferes with the adrenocortical function (4), and

probenecid interferes with the steady-state concentrations of brain TP (5).

In our report we followed the approximation of the conversion index and that of  $k_3$ , with their inherent limitations. We maintain that the rate of 5-HT turnover in the brains of mice with a high degree of tolerance to, and physical dependence on, morphine can remain unchanged. We do not exclude the possibility that one can find special strains of mice and experimental conditions in which one can obtain a change of 5-HT turnover rate associated with morphine dependence. However, the fact that we (1) and others (6) have found no association between increased turnover of brain 5-HT and physical dependence on morphine makes the idea of a causal relationship between the two phenomena highly improbable.

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#### References

1. D. L. Cheney, A. Goldstein, S. Algeri, E. Costa, *Science* **171**, 1169 (1971).
2. E. Costa, A. R. Green, S. H. Koslow, H. F. LeFevre, A. U. Revuelta, C. Wang, *Pharmacol. Rev.* **24**, 167 (1972).
3. E. C. Azmitia, Jr., S. Algeri, E. Costa, *Science* **169**, 201 (1970); G. C. Sedvall, V. K. Weise, I. J. Kopin, *J. Pharmacol. Exp. Ther.* **159**, 274 (1968).
4. A. Tagliamonte, P. Tagliamonte, J. Perez-Cruet, S. Stern, G. L. Gessa, *J. Pharmacol. Exp. Ther.* **177**, 475 (1971).
5. L. J. Weber and A. Horita, *Biochem. Pharmacol.* **14**, 1141 (1965).
6. P. J. Schechter, W. Lovenberg, A. Sjoerdsma, *ibid.* **21**, 751 (1972); I. Marshall and D. C. Grahame-Smith, *J. Pharmacol. Exp. Ther.* **179**, 634 (1971); D. R. Haubrich and D. E. Blake, *Fed. Proc.* **28**, 793 (1969); S. Algeri and E. Costa, *Biochem. Pharmacol.* **20**, 877 (1971).

30 August 1972

## The Structure of Morphine Monohemisuccinate

We have recently described the preparation and immunogenic properties of a conjugate of morphine 3-hemisuccinate with bovine serum albumin (1). We now have chemical evidence that the attachment of the succinoyl moiety to morphine is through the 6- rather than the 3-hydroxyl group and that the conjugate with bovine serum albumin (BSA) should therefore be similarly formulated.

We originally reported that a ferric chloride test on the succinoyl derivative (1) was negative. This observation has now been found to be in error; indeed 1 does give a positive test with  $\text{FeCl}_3$ , an indication that a free phenolic group is present at C-3 and that the

succinoyl moiety must therefore be attached to the alcoholic hydroxyl at C-6. Rigorous proof was obtained as follows. Methylation of 1 with diazomethane in ether with a trace of methanol for 12 hours gave a 62 percent yield of a methyl ester methyl ether of 1 together with 7 percent of codeine, separated by thin-layer chromatography on silica gel with a solvent system composed of ethyl acetate, methanol, and ammonium hydroxide (17 : 2 : 1). The above methyl ester methyl ether on hydrolysis with 2 percent KOH in methanol at 25°C for 1 hour yielded, after one recrystallization from hexane, codeine (54 percent), which melted at 151° to 153°C and exhibited infrared