activity (*16*). In the liver of diabetic rats, insulin causes a sixfold increase in glycogen phosphatase activity (*17*), a twofold increase in glycogen synthase activity (*17*), and a fivefold increase in albumin synthesis (*18*). The latter effect is accompanied by a threefold increase in albumin mRNA (*18*). Our data therefore demonstrate the most pronounced inductive effect by insulin on a specific gene product. It is not known, however, whether insulin changes pancreatic amylase mRNA by stimulating its synthesis or inhibiting its degradation. The apparently long lag period required for this inductive effect raises the question whether insulin acts directly or indirectly an amylase gene transcription. Studies on cellular systems, in vitro, may provide conclusive answers to these questions. Because of the dramatic effects on a major gene product, the pancreatic acinar cell may be a useful model system for studying the molecular actions of insulin.

MURRAY KORC*
Cell Biology Laboratory, Mount Zion Hospital and Medical Center and *Department of Medicine, University of California, San Francisco 94143*
DAVID OWERBACH†
CARMEN QUINTO
WILLIAM J. RUTTER
Department of Biochemistry and Biophysics, University of California, San Francisco 94143

References and Notes

1. J. R. Henderson, *Lancet* **1969-II**, 469 (1969).
2. F. Malaisse-Lagae, M. Ravazzola, P. Robberecht, A. Vandermeers, W. J. Malaisse, L. Orci, *Science* **190**, 795 (1975).
3. R. Pictet and W. J. Rutter, in *Handbook of Physiology-Endocrinology*, D. F. Steiner and N. Freinkel, Eds. (American Physiological Society, Washington, D.C., 1972), vol. 1, pp. 25–65.
4. M. Korc, Y. Iwamoto, H. Sankaran, J. A. Williams, I. D. Goldfine, *Am. J. Physiol.* **240**, G56 (1981).
5. J. C. Palla, A. Ben Abdellil, P. Desnuelle, *Biochim. Biophys. Acta* **158**, 25 (1968).
6. J. T. Snook, *Am. J. Physiol.* **215**, 1329 (1968).
7. H. O. Soling and K. O. Unger, *Eur. J. Clin. Invest.* **2**, 199 (1972).
8. M. Korc, J. A. Williams, I. D. Goldfine, *Intern. Med.* **1**, 20 (1981).
9. W. Y. Chey, H. Shay, C. R. Shuman, *Ann. Intern. Med.* **59**, 812 (1964).
10. W. Domschke, F. Tympner, S. Domschke, L. Demling, *Digest. Dis.* **20**, 309 (1975).
11. M. W. Balk, C. M. Lang, W. J. White, B. L. Munger, *Lab. Invest.* **32**, 28 (1975).
12. B. M. Frier, O. K. Faber, C. Binder, H. L. Elliott, *Diabetologia* **14**, 301 (1978).
13. A. Danielsson, *Digestion* **10**, 150 (1974).
14. R. J. MacDonald, M. M. Crerar, W. F. Swain, R. L. Pictet, G. Thomas, W. J. Rutter, *Nature (London)* **287**, 117 (1980).
15. C. Quinto, M. Quiroga, W. F. Swain, P. Valenzuela, W. J. Rutter, in preparation.
16. L. G. Roger and R. E. Fellows, *Endocrinology* **106**, 619 (1980).
17. T. B. Miller, Jr., *Biochim. Biophys. Acta* **583**, 36 (1979).
18. D. E. Peavy, J. M. Taylor, L. S. Jefferson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5879 (1978).
19. J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **24**, 5294 (1979).
20. M. Mevarech, B. E. Noyes, K. L. Agarwal, *J. Biol. Chem.* **254**, 7472 (1979).
21. P. W. J. Rigby, M. Dieckmann, C. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 237 (1977).
22. J. Rinderknecht, P. Wilding, B. J. Havenback, *Experientia* **23**, 805 (1967).
23. B. F. Erlanger, F. Edel, A. G. Cooper, *Arch. Biochem. Biophys.* **115**, 206 (1966).
24. D. Denhardt, *Biochem. Biophys. Res. Commun.* **23**, 641 (1966).
25. G. M. Wahl, M. Stern, G. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3683 (1979).
26. R. J. MacDonald, unpublished result.
27. Supported by NIH grant AM 21344 (to W.J.R.) and awards (to M.K.) from the Juvenile Diabetes Foundation, and from the Committee on Research, San Francisco division of the Academic Senate of the University of California; C.Q. was supported by Centro de Fijacion de Nitrogeno, Universidad Autonoma de Mexico.
* Present address: Department of Medicine, University of Arizona, Health Sciences Center, Tucson 85724.
† Present address: Hagedorn Research Laboratory, Niels Steensensvej 6, DK-2820 Gentofte, Denmark.

2 December 1980; revised 17 April 1981

Tonic Immobility Produces Hyperalgesia and Antagonizes Morphine Analgesia

Abstract. *Hyperalgesia was demonstrated during and immediately after termination of tonic immobility in the lizard* Anolis carolinensis. *Additionally, tonic immobility antagonized morphine-induced analgesia. In conjunction with other research, these data suggest that the response is accompanied by a reduced availability of serotonin, possibly at postsynaptic receptors of raphe neurons.*

Tonic immobility (TI) is a catatonic-like state of behavioral inhibition elicited by brief physical restraint exhibited in a wide variety of species including some mammals, fowl, reptiles, fish, and insects (*1*). In a typical laboratory setting, an animal restrained on its side or back will cease struggling after a few seconds. After restraint is removed, the animal will remain immobile for periods varying from a few seconds to well over an hour. The response is characterized by inhibition of movement, waxy flexibility, intermittent eye closures, and Parkinsonian-like tremors. Tonic immobility is very sensitive to manipulations involving fear, and it has been proposed as a predator defense mechanism of last resort (*1*). Gallup and Maser (*2*) have suggested a possible connection between TI and human catalepsy and catatonia.

Of primary interest to researchers are the neural mechanisms underlying TI. The model most strongly supported has been the serotonergic-midbrain raphe model proposed by Wallnau and Gallup (*3*). The focus of this model is the inverse relationship between drug-induced effects on TI durations and their effects on activity of the raphe nuclei. Presumably, administration of serotonin (5HT) or its agonists results in excess 5HT at postsynaptic receptors producing inhibitory feedback to the raphe, which in turn produces concomitant increases in TI durations. In light of this model, the apparent relationship between 5HT, the raphe, and nociception (*4, 5*) makes the nature of pain sensitivity during TI an interesting (yet essentially unanswered) question. Enhancing the significance of this question is the possibility that endogenous release of opiate peptides (endorphins and enkephalins) may be involved in TI mechanisms (*6*). Despite the theoretical appeal of such information, researchers have previously found quantification of nociception difficult in animals that exhibit the TI response.

We now report the lizard *Anolis carolinensis* to be an excellent preparation for the objective study of pain sensitivity accompanying TI. We found the anole lizard, which has previously been used in TI research (*7, 8*), well suited for tests in a tail-flick apparatus. Tail-flick latencies for lizards tested 15 seconds after induction of TI with their tails positioned in the apparatus were compared with those tested after comparable handling. The TI-treated lizards demonstrated increased sensitivity to noxious stimuli as evidenced by significantly shorter tail-flick latencies [1.667 and 2.212 seconds, respectively; $F(1, 28) = 11.38$, $P < .005$]. Control lizards were also compared with those tested immediately after either 15 or 30 seconds of TI, which was terminated by the experimenter. Again, TI-treated lizards exhibited shorter tail-flick latencies [$F(2, 57) = 4.686$, $P < .02$]. Thus, lizards were more sensitive (*9*) to noxious stimuli during and immediately after TI was externally terminated. An earlier, subjective report of analgesia during TI in rabbits (*10*) suggests that these effects may not generalize to that species. The subjective nature of this report leaves the question of generalization of TI effects to rabbits relatively unresolved (*11*).

The TI-induced changes in nociception reported here are quite similar to effects produced by depletion of 5HT (*7*). *para*-Chlorophenylalanine depletion of 5HT or lesions of the 5HT-rich raphe nuclei produce hyperalgesia as well as antagonism of morphine analgesia (*5*). The similarities between these independent findings and our data suggest that

0036-8075/81/0717-0353$00.50/0

TI may be accompanied by reduced availability of 5HT at the raphe. To further test this hypothesis, we tested the effects of TI induction on morphine analgesia.

Lizards treated with morphine (5 mg per kilogram of body weight, injected intraperitoneally) or saline were tested in the tail-flick apparatus 10 minutes after injection, either during TI or after comparable handling. The induction of TI blocked the analgesic effect of morphine, as did 5HT depletion (Fig. 1). In addition, both TI groups were hyperalgesic relative to the non-TI groups.

This final test further demonstrates that TI produces changes in nociception identical to those produced by depletion of 5HT. Evidence suggests that an inhibitory serotonergic pathway between the raphe and other brain locations is involved in morphine analgesia (*12*). Apparently, increased activity of raphe neurons (either by electrical stimulation or systemic administration of morphine) inhibits pain-evoked spikes in the spinal cord and locus coeruleus, effects that are reversed by 5HT depletion. Such data support further the connection between the proposed serotonergic mechanisms and the mechanisms of morphine analgesia. Synthesis of our data, in conjunction with an earlier report that repeated induction of TI reduces brain 5HT (*13*), suggests that reduced postsynaptic availability of 5HT at the raphe accompanies TI. This view is inconsistent with the original serotonergic model of Wallnau and Gallup. Within that model, reduced raphe activity resulting from feedback from excess postsynaptic 5HT facilitated TI durations.

The notion of decreased postsynaptic 5HT availability during TI is consistent with a recent revision of the serotonergic model proposed by Boren *et al.* (*14*). This revised model links drug-induced changes in TI not to their effects on raphe activity, but to their effects on postsynaptic serotonergic receptors. Treatments decreasing stimulation of these receptors result in increased TI durations, whereas increased stimulation shortens TI durations. These effects can be independent of raphe activity. The basis for this modification is the demonstration that very large increases of 5HT actually shorten TI durations, even though raphe activity is reduced. For example, combined administration of two 5HT agonists (tryptophan and pargyline) greatly shorten TI durations, although when administered separately each has the opposite effect (*14*). The administration of 5HT also has biphasic effects on TI (*15*): whereas relatively low doses lengthen TI, larger doses have no effect, and very large doses actually shorten TI durations (*16*). Recent physiological evidence suggests that 5HT inhibits the raphe by direct action on the neuronal membrane rather than by negative feedback from postsynaptic receptors (*17*). Thus, drugs that reduce raphe activity also reduce 5HT output to postsynaptic receptors by this direct inhibition and thereby increase TI durations. If these drugs produce enough excess 5HT to directly stimulate postsynaptic receptors, however, the effect of reduced raphe activity is counteracted and shortened TI durations are produced.

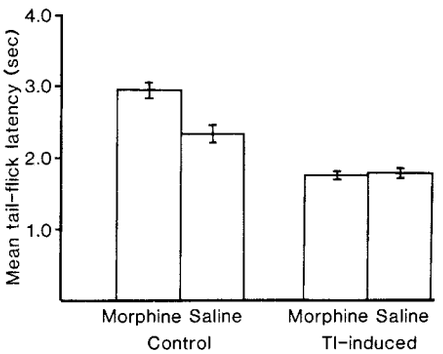

Fig. 1. Mean tail-flick latencies for lizards treated with morphine (5 mg/kg, intraperitoneally) or saline and tested normally for 15 seconds after induction of TI. Results indicate that morphine produced reliable analgesia relative to the saline control ($P < .05$) when tested normally but that there was no significant difference between lizards injected with morphine and saline when tested after TI. Further, both groups tested after TI exhibited reliably shorter latencies than the saline ($P < .05$) and morphine ($P < .01$) groups tested normally.

The notion of opiate peptide involvement in TI gained much support from the demonstrations of opiate-induced increases in TI durations (*6*). Depletion of 5HT eliminates morphine enhancement of TI (*18*), suggesting that these opiate effects are mediated by serotonin-containing systems. Opiate peptide analogs essentially without direct opiate activity also increase TI durations (*19*), further implicating nonnarcotic action. Our demonstration of hyperalgesia during TI supports serotonergic systems rather than direct opiate involvement as the source of the effects.

The Boren *et al.* proposal that effects on postsynaptic serotonergic receptors are the critical focus of drug-induced changes in TI durations (*14*) appears to be the best account for the TI literature to date. The synthesis of our demonstration of changes in pain sensitivity with independent work concerning 5HT, the raphe, and nociception suggests mechanisms consistent with this model. The postsynaptic 5HT model, like other models, is based on measurement of TI durations (and often number of trials required to induce the response). Our data represent indirect evidence, independent of duration measurement, supporting what appears to be the most accurate of these models. Although direct evidence has yet to be produced, support from two different approaches adds credence to the hypothesis that decreased excitation of postsynaptic serotonergic receptors to raphe neurons is an integral part of the neural mechanisms of tonic immobility.

MICHAEL D. MAUK
RICHARD D. OLSON
GERALD J. LAHOSTE
GAYLE A. OLSON

Department of Psychology, University of New Orleans, New Orleans, Louisiana 70122

References and Notes

1. G. G. Gallup, Jr., *Psychol. Bull.* **81**, 836 (1974).
2. ——— and J. D. Maser, in *Psychopathology: Experimental Models*, J. D. Maser and M. E. P. Seligman, Eds. (Freeman, San Francisco, 1977).
3. L. B. Wallnau and G. G. Gallup, Jr., *Biobehav. Rev.* **1**, 35 (1977).
4. E. Genovese, N. Zonta, P. Mantegazza, *Psychopharmacologia* **32**, 359 (1973); S. S. Tenen, *ibid.* **10**, 204 (1967); J. F. W. Deakin and J. O. Dostrovsky, *Br. J. Pharmacol.* **63**, 159 (1978).
5. R. Samanin, D. Ghezzi, C. Macron, L. Valzelli, *Psychopharmacologia* **33**, 365 (1973); W. T. Chance, G. M. Krynock, J. A. Rosencrans, *ibid.* **56**, 133 (1978); T. D. Oleson, D. A. Twimbly, J. C. Liebeskind, *Pain* **4**, 211 (1978); A. H. Dickenson, J. L. Oliveras, J. M. Besson, *Brain Res.* **170**, 95 (1979); M. M. Behbehani and S. L. Pomeroy, *ibid.* **149**, 266 (1978); G. Urca and J. C. Liebeskind, *ibid.* **161**, 162 (1979).
6. G. Carli, *Psychol. Rec.* **1**, 123 (1977).
7. D. G. Webster, T. H. Lanthorn, M. E. Meyer, *Physiol. Psychol.* **7**, 451 (1979); D. H. Edson and G. G. Gallup, Jr., *Psychonom. Sci.* **26**, 27 (1972).
8. C. W. Hennig, W. P. Dunlap, C. T. Harston, *Physiol. Behav.* **22**, 1079 (1979).
9. Animal pain research is plagued by the inability to distinguish pain sensitivity from pain reactivity. We sidestep this issue in that whether we have measured sensitivity or reactivity is less important than the fact that our data parallel the other findings.
10. W. S. Rapson and T. C. Jones, *Lab. Anim. Care* **14**, 131 (1964).
11. D. C. Hatton, R. Tankle, T. Lanthorn, M. E. Meyer, *Behav. Biol.* **24**, 97 (1978).
12. M. Segal, *J. Physiol. (London)* **286**, 401 (1979); T. D. Oleson and J. C. Liebeskind, *Physiologist* **18**, 338 (1975).
13. W. T. Liberson, J. Bernsohn, A. Wilson, V. Daly, *J. Neuropsychiatry* **5**, 363 (1964).
14. J. L. Boren *et al.*, *Pharmacol. Biochem. Behav.* **11**, 17 (1979).
15. C. W. Hennig, *ibid.* **12**, 519 (1980).
16. J. D. Maser, G. G. Gallup, Jr., L. E. Hicks, *J. Comp. Physiol. Psychol.* **89**, 319 (1975).
17. G. K. Aghajanian and R. Y. Wong, *Brain Res.* **122**, 229 (1977); S. S. Mosko, D. Haubrich, B. L. Jacobs, *ibid.* **119**, 269 (1977); G. K. Aghajanian and R. Y. Wong, in *Psychopharmacology: A Generation of Progress*, M. A. Lipton *et al.*, Eds. (Raven, New York, 1978); G. K. Aghajanian, W. E. Foote, M. H. Sheard, *Science* **161**, 706 (1968); G. K. Aghajanian, H. J. Haigler, F. E. Bloom, *Life Sci.* **11**, 615 (1972).
18. L. E. Hicks, J. D. Maser, G. G. Gallup, Jr., P. H. Edson, *Psychopharmacologia* **42**, 51 (1974); L. B. Wallnau and G. G. Gallup, Jr., *Pharmacol. Biochem. Behav.* **10**, 499 (1978).
19. R. D. Olson, A. J. Kastin, G. J. LaHoste, G. A. Olson, D. H. Coy, *Pharmacol. Biochem. Behav.* **11**, 705 (1979).
20. We thank A. J. Kastin for helpful suggestions during the study.

20 March 1981