tivity, and specificity. The application of radioreceptor assay makes clear, for the first time, that immunoreactive HGH comprises components that have different capacities to displace labeled HGH from specific binding sites on viable cells. It can now be determined whether these factors are important in pathophysiologic states of growth hormone action.

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Dopamine Synthesis: Stimulation by a Hypothalamic Factor

Abstract. *The effect of treatment with the factor that inhibits the release of melanocyte stimulating hormone (MSH) identified as 1-prolyl-1-leucylglycinamide (MIF) on brain catecholamine synthesis was examined in normal and hypophysectomized rats. The tripeptide induced a dose-related increase in striatal dopamine synthesis in slices obtained from treated normal animals but not in hypophysectomized animals. Hypothalamic norepinephrine synthesis was unaltered by MIF treatment in normal as well as in hypophysectomized rats. In addition, dopamine and norepinephrine syntheses were depressed in untreated hypophysectomized animals, as compared to normal controls. These results constitute the first direct demonstration of a central neurochemical effect of a hypothalamic factor.*

The physiological role of melanocyte stimulating hormone (MSH) in mammals has not been established. Some extrapigmentary functions have been suggested (*1*, *2*); the hormone produces anxiety, motor restlessness and alterations in electroencephographs in humans (*2*), and exacerbation of symptomatology in patients suffering from Parkinsonism (*3*).

Release of MSH from the pars intermedia of the pituitary is controlled by hypothalamic releasing and release-inhibiting factors (*4*). A possible inhibiting factor of MSH release (MIF) has been isolated from mammalian hypothalamus and identified as the tripeptide, 1-prolyl-l-leucylglycinamide (*5*).

Recent pharmacological evidence has suggested a direct central role for MIF. Plotnikoff and his co-workers have shown that prior treatment with MIF potentiates the behavioral effects of L-dopa (*6*), antagonizes the central and peripheral effects induced by oxotremorine (*7*), and reverses the sedative effects of reserpine in mice and monkeys (*8*). These actions of 1-prolyl-1-leucylglycinamide suggest a possible anti-Parkinsonian or antidepressant effect through an interaction with brain dopaminergic neuronal systems. Accordingly, we studied the effect of MIF on brain dopamine metabolism.

Table 1. Concentrations of endogenous striatal dopamine and hypothalamic norepinephrine in rats treated with various single and repeated doses of MIF. Rats were killed 1.5 hours after the last injection of MIF. Each value is the mean of eight brain areas ± the standard error of the mean.

MIF (mg/kg)	Striatal dopamine (μg/g)	Hypothalamic norepinephrine (μg/g)
Saline only	4.23 ± .05	1.84 ± .02
0.5	4.12 ± .04	1.81 ± .02
1.0	4.09 ± .03	1.69 ± .03
5.0	4.21 ± .04	1.78 ± .02
4 days of 4.0	5.29 × .05*	1.73 ± .03
4 days of 1.0	4.94 × .02*	1.83 ± .03

* $P < .01$.

Normal or hypophysectomized male Sprague-Dawley rats (2 to 4 weeks after operation) housed under a regime of 12 hours of light and 12 hours of darkness (LD 12 : 12) given chow and water (normal animals) or 5.0 percent dextrose (hypophysectiomized animals) were used. Synthetic MIF (Abbott-40509) was dissolved in saline and administered intraperitoneally. Control rats were injected with an equal volume of saline. All injections were made between 2 and 4 hours after the beginning of the light cycle; the rats were killed 1.5 hours after receiving the injections. The brains were quickly removed and rinsed in ice-cold Krebs-Henseleit physiological solution; the hypothalamus and striatum were dissected out for determination of endogenous tyrosine, dopamine, and norepinephrine. The areas to be analyzed were weighed and homogenized in 0.4*N* perchloric acid, and the acid extract was poured over columns containing Dowex 50 × 4 (K^+ form). Tyrosine, dopamine, and norepinephrine were subsequently eluted with *p*H 4.5 buffer, 0.4*N* HCl, and 4*N* HCl, respectively, according to the method of Neff *et al.* (*9*). Eluates were further purified by either passing through the alumina (tyrosine) or by being adsorbed onto the alumina at *p*H 8.4, with subsequent elution (of norepinephrine and dopamine) with 0.2*N* HCl. Endogenous norepinephrine and dopamine were assayed fluorimetrically according to the methods of Anton and Sayre (*10*) and Laverty and Taylor (*11*), respectively.

Catecholamine synthesis was studied in hypothalamic and striatal slices from rats treated with MIF and saline. Brain slices were obtained with the use of a mechanical McIlwain tissue chopper (set on 0.4 mm) and placed in 25-ml flasks containing 2 ml of oxygenated, cold Krebs-Henseleit solution. Incubations were carried out in a metabolic shaker at 37°C in an atmosphere consisting of 95 percent O_2 and 5 percent CO_2. After an initial incubation period of 10 minutes 50 μl of [3,5-^{3}H]tyrosine was added to each sample to give a final concentration of $8.15 \times 10^{-6} M$ tyrosine and the incubation was continued for an additional 45 minutes. Tissue and media were rapidly separated by filtration. The tissue was washed twice with 2 ml of cold physiological solution, and the washings were added to the media. The tissue was homogenized in 0.4*N* perchloric acid. The media samples were adjusted to a final concentration of 0.4*N*

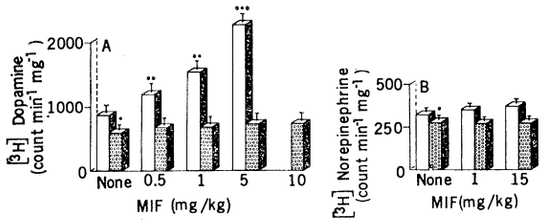

Fig. 1. [^{3}H]Dopamine and [^{3}H]norepinephrine syntheses in striatal and hypothalamic slices obtained from saline- and MIF-treated rats, respectively. Slices were obtained from normal (open bars) and hypophysectomized (hatched bars) rats. Each bar represents the mean ± S.E.M. of ten determinations. $*P < .05$; $**P < .005$; $***P < .001$.

perchloric acid. Unlabeled tyrosine, norepinephrine, and dopamine were added as carriers to all samples. [^{3}H]-Tyrosine, [^{3}H]dopamine, and [^{3}H]norepinephrine were isolated as described above on Dowex columns and portions of their respective eluates were added to scintillation fluid for counting.

Figure 1 shows the effect of MIF on the synthesis of [^{3}H]dopamine from [^{3}H]tyrosine in striatal slices obtained from normal and hypophysectomized rats. In doses of 0.5, 1, and 5 mg/kg, MIF increased dopamine synthesis in normal but not in hypophysectomized animals. The synthesis of [^{3}H]norepinephrine in hypothalamic slices from normal and hypophysectomized rats was not altered by treatment with MIF. Furthermore, dopamine and norepinephrine syntheses were 28 and 18 percent lower in untreated hypophysectomized rats, as compared to control animals, respectively. The specific activity of tyrosine in striatal and hypothalamic brain slices was unaltered by MIF treatment in both normal and hypophysectomized animals. Endogenous concentrations of dopamine (striatum) and norepinephrine (hypothalamus) were unchanged by single injections with MIF in normal animals (Table 1). However, repeated daily administration of MIF for 4 days induced an increase in striatal dopamine concentrations. Rectal temperature was not significantly altered by treatment with MIF in normal as well as in hypophysectomized rats.

Our data show that MIF exerts a stimulatory influence on striatal dopamine synthesis while not affecting hypothalamic norepinephrine synthesis in the rat. The influence of MIF on dopamine synthesis appears to be mediated by the pituitary because prior hypophysectomy prevents this MIF effect. However, it is not possible to unequivocally ascribe this action of MIF solely to its influence on MSH release because other pituitary hormones whose influence is eliminated by the hypophysectomy may also interact with brain dopamine and so mask the MIF effect. The complex interaction of pituitary hormones with brain catecholamine synthesis is also supported by our finding of reduced brain catecholamine synthesis in hypophysectomized rats. Furthermore, the release of leuteinizing hormone (LH), follicle stimulating hormone (FSH), growth hormone, and prolactin from the pituitary has been shown to be controlled by brain dopaminergic mechanisms (*12*), mediated by hypothalamic release-inhibiting or -stimulating factors (*13*). Brain catecholamine neurons may also be part of a feedback system that controls the secretion of pituitary hormones. Hokfelt and Fuxe (*14*) have demonstrated that prolactin, but not LH, FSH, adrenocorticotrophic hormone, or antidiuretic hormone ADH, induces an increase in the turnover of median eminence dopamine.

The release of MSH has also been shown to be mediated by catecholaminergic mechanisms (*15*). Taleisnik *et al.* (*15*) have shown that the hypothalamic content of MIF is influenced by catecholamines, that interference with hypothalamic adrenergic transmission is associated with an increase in MSH release, but that implantation of catecholamines in the ventricular system of the rat results in prevention of MSH release.

Pharmacological studies with MSH in Parkinsonian patients and with MIF in rats support the hypothesis that MSH induces a reduction, while MIF induces an activation, of brain dopaminergic transmission. These studies indicated possible anti-Parkinsonian and antidepressant activity for MIF, so much so as to warrant clinical trials with MIF (*16*). Plotnikoff *et al.* (*6*) have observed potentiation of the behavioral effects of L-dopa in normal as well as in hypophysectomized mice given prior treatment with MIF.

This study suggests either a dopaminergic action for MIF or its interaction with L-dopa, which is independent of pituitary mediation. The ineffectiveness of MIF in inducing dopamine synthesis in hypophysectomized animals observed in our study suggests that the L-dopa potentiation by MIF resulted from an interaction between MIF and L-dopa. It is also possible that MIF-induced stimulation of dopamine synthesis from tyrosine is dependent on an intact pituitary, while the conversion of L-dopa to dopamine in MIF-treated animals is not dependent on pituitary hormonal output.

In conclusion, we have presented the first direct neurochemical evidence that a hypothalamic hormone exerts an effect on the central synthesis of the neurotransmitter, dopamine. The marked increase in synthesis affected by MIF is specific to dopaminergic neurons, as MIF does not affect norepinephrine synthesis in the hypothalamus. Furthermore, we show that hormonal imbalance resulting from hypophysectomy induces a decrease in striatal dopamine, as well as in hypothalamic norepinephrine, syntheses.

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