extensible throat fan supported by hyoid cartilages. The dewlap is secondarily reduced or lost in several unrelated species groups (*12*). Among the species of the *monticola* group *Anolis christophei* has a large dewlap and uniform squamation and lacks a bold body pattern, all characters suggesting that it is the most primitive species of the group (*13*); *A. christophei* again has the $2n = 36$ karyotype. *Anolis monticola* has a reduced dewlap, non-uniform squamation, and a bold ocellate body pattern. It is clearly a highly derived form.

The diploid number of 48 observed in *A. monticola* could be derived from the primitive $2n = 36$ karyotype by centric fission of the metacentric macrochromosomes. The telocentric morphology of many of the macrochromosomes in the *A. monticola* karyotype is compatible with a fission hypothesis. That the alternative explanation—the *monticola* karyotype is a retained primitive condition—is implausible can be seen in Fig. 2. If the high diploid number is ancestral, at each point marked by an × there must have been an independent evolution of a diploid number of 36. Even if this event occurred the requisite 12 times, it is extremely unlikely that the resulting karyotypes would be as uniform in chromosome morphology as they are known to be. If the diploid number of 36 is accepted as ancestral (*10*, *14*, *15*), then fission need be invoked at only two points in the phylogeny, marked by a +. Non-telocentric chromosomes in the karyotype of *A. monticola* are easily interpreted as fission products modified by pericentric inversion. The polymorphism for diploid number in *A. monticola* can be interpreted as secondary centric fusion or incomplete stabilization of a fully fissioned karyotype.

Single or multiple fissions have been advanced as the probable evolutionary pathway to a number of lizard karyotypes. Included are members of the families Teiidae (*15*), Anguidae (*16*), and Iguanidae: *Plica plica* (*10*), *Anolis oculatus* (*7*), and *Sceloporus grammicus* (*17*). The occurrence of multiple fissions in the evolution of the karyotype of *A. monticola* strengthens these interpretations.

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16 March 1972

Dopamine: Mediator of Brain Polysome Disaggregation after L-Dopa

Abstract. *The disaggregation of brain polysomes which is produced by giving large doses of* L-*dopa to rats is not reproduced by administering its metabolite, 3-*O*-methyldopa, by giving* D-*dopa, which also depletes the brain of* S*-adenosylmethionine but is not converted to catecholamines, or by giving the* L-*dopa after a decarboxylase inhibitor. Polysome disaggregation is potentiated by the prior administration of a monoamine oxidase inhibitor, indicating that formation of a catecholamine is an obligatory requirement. These observations suggest that the mechanism by which* L-*dopa disaggregates brain polysomes involves its conversion to dopamine within the majority of brain cells.*

Administration of L-dopa (500 mg per kilogram of body weight, intraperitoneally) to rats is followed between 40 and 60 minutes by the disaggregation of polysomes obtained from whole brain. This disaggregation is unaccompanied by a decrease in the concentrations of any free amino acid; brain tryptophan levels actually increase significantly (*1*).

We have attempted to identify L-dopa or one of its metabolites as the agent causing disaggregation of brain polysomes. Administered L-dopa is transformed by the brain to the catecholamines dopamine and norepinephrine, and their metabolites, and to the amino acid 3-*O*-methyldopa (Fig. 1) (*2*). The *O*-methylation of exogenous dopa, catalyzed by catechol *O*-methyltransferase, depletes the brain of *S*-adenosylmethionine (*3*). To identify which of the compounds formed or utilized after administration of dopa is involved in polysome disaggregation, we examined the state of polysome aggregation in animals given (i) 3-*O*-methyldopa; (ii) D-dopa, which forms 3-*O*-methyldopa in the brain but does not undergo decarboxylation to dopamine; (iii) L-dopa along with an inhibitor (RO4-4602) of its decarboxylation to dopamine; (iv) L-dopa along with an inhibitor (pheniprazine) of monoamine oxidase; or (v) intracisternal dopamine or norepinephrine. Changes in whole brain polysome aggregation were correlated with alterations in the concentrations of dopa, 3-*O*-methyldopa, *S*-adenosylmethionine, dopamine, and norepinephrine in the brain.

Male Sprague-Dawley rats (Charles River Laboratories) weighing approximately 50 g were exposed to light from 9 a.m. to 9 p.m. daily. They were housed four per cage and given free access to Purina Chow and water. L-Dopa, 3-*O*-methyldopa, and D-dopa (Hoffmann-LaRoche, Inc.) were dissolved in 0.05*N* HCl and administered intraperitoneally; control animals received only the acidic diluent. The decarboxylase inhibitor RO4-4602 (Hoffmann-LaRoche, Inc.) and the monoamine oxidase inhibitor pheniprazine (JB 516; Lakeside Laboratories, Inc.) were dissolved in water and administered intraperitoneally. Dopamine hydrochloride

Table 1. Effects of L-dopa and related drugs on brain polysome profiles and dopa metabolism of 50-g male rats. Data are presented as mean and standard error of the mean. Significance of differences was evaluated by Student's *t*-test. The number of determinations in each group is given in parentheses. If two determinations were made for a group, the two values are given in brackets. Abbreviations: i.p., intraperitoneal; i.c., intracisternal.

Treatment	Route of administration	Dose (mg/kg)	Time after injection (min)	Polysomes (percent of profile)	Dopa (μg/g)	3-*O*-methyldopa (μg/g)	*S*-Adenosylmethionine (percent of control)*	Dopamine (ng/g)	Norepinephrine (ng/g)
Control	i.p.	0	60	65 ± 1.9 (8)	< 0.1 (11)	< 0.1 (9)			
L-Dopa	i.p.	500	40		18.7 ± 4.3 (7)†	0.5 ± 0.1 (7)†			
			60	43 ± 3.6 (7)†	6.8 ± 2.0 (7)†	1.2 ± 0.4 (8)‡			
3-*O*-methyldopa	i.p.	500	60	60 [64, 56]	0.3 ± 0.1 (7)§	4.6 ± 1.9 (7)§			
			120	65 [67, 63]	0.3 ± 0.05 (6)†	11.8 ± 1.9 (8)†			
Control	i.p.	0	60		< 0.1 (6)	< 0.1 (6)	100 ± 12.4 (6)	505 ± 32 (6)	300 ± 19 (6)
L-Dopa	i.p.	500	60		9.5 ± 1.8 (6)†	3.4 ± 0.4 (6)†	32 ± 7.9 (6)†	2,230 ± 423 (5)‡	330 ± 22 (6)
D-Dopa	i.p.	500	60	61 ± 4.0 (3)	10.7 ± 0.8 (6)†	2.9 ± 0.1 (6)†	27 ± 5.0 (6)†	640 ± 47 (6)	310 ± 23 (6)
RO4-4602	i.p.	800	90	65 ± 4.4 (3)	2.9 ± 1.0 (6)§	0.1 ± 0.02 (6)	45 ± 10.3 (6)‡	346 ± 34 (6)‡	188 ± 10 (6)†
RO4-4602 plus L-dopa	i.p.	800 500	90 60	60 ± 2.1 (3)	40.2 ± 2.5 (6)†	4.3 ± 0.9 (6)†	39 ± 4.0 (6)‡	298 ± 50 (6)‡	200 ± 10 (6)†
Control	i.p.	0	60	73 [73, 74]	< 0.1 (5)			503 ± 90 (5)	305 ± 29 (4)
L-Dopa	i.p.	100	60	75 ± 3.1 (3)	2.3 ± 0.8 (6)†			780 ± 24 (6)§	348 ± 12 (6)
Pheniprazine	i.p.	10	180	62 ± 1.5 (4)	< 0.1 (5)			779 ± 92 (5)	398 ± 20 (5)§
Pheniprazine plus L-dopa	i.p. i.p.	10 50	180 60		0.3 ± 0.03 (5)†			1,860 ± 185 (4)†	521 ± 24 (5)†
Pheniprazine plus L-dopa	i.p. i.p.	10 100	180 60	32 ± 7.3 (4)†	1.2 ± 0.2 (5)†			2,890 ± 397 (5)†	618 ± 63 (5)‡
Pheniprazine plus L-dopa	i.p. i.p.	10 150	180 60		1.3 ± 0.1 (3)†			7,940 [7,910; 7,980]†	691 ± 67 (3)‡
Pheniprazine plus L-dopa	i.p. i.p.	10 500	180 60	41 ± 9.0 (3)‡	17.0 ± 2.4 (4)†			30,000 ± 2,310 (4)†	801 ± 63 (4)†
		(micrograms per rat)							
Control	i.c.	0	45	60	< 0.1 (3)	< 0.1 (3)	100 ± 22.6 (3)	470 ± 34 (3)	289 ± 20 (3)
Dopamine	i.c.	100	15	59 [60, 57]	< 0.1 (5)	< 0.1 (5)	92 ± 2.6 (5)	6,020 ± 1,260 (5)§	308 ± 32 (5)
			45	58 [60, 56]	< 0.1 (5)	< 0.1 (5)	86 ± 1.7 (5)	903 ± 390 (5)	212 ± 18 (5)§
Norepinephrine	i.c.	100	15	57 [63, 52]	< 0.1 (5)	< 0.1 (5)	94 ± 4.6 (5)	555 ± 26 (5)	20,800 ± 2,470 (5)†
			45	54 [52, 57]	< 0.1 (5)	< 0.1 (5)	82 ± 6.7 (5)	593 ± 53 (5)	10,200 ± 826 (5)†

* Control brains contained an average of 13.4 μg of *S*-adenosylmethionine per gram. † $P < .001$ differs from control group. ‡ $P < .01$ differs from control group. § $P < .05$ differs from control group.

and norepinephrine (Regis Chemical Co.) were dissolved in 0.05*N* HCl and administered intracisternally; animals received one of the catecholamines or only the vehicle.

In all experiments, animals were decapitated at 1 p.m., and brain polysome profiles were prepared (*1*). For each determination of a profile, two whole brains were pooled, and the profiles were run in duplicate. For the metabolic assays, single brains were homogenized in 10 ml of 10 percent trichloroacetic acid in 0.05*N* HCl. After centrifugation at 10,000*g*, samples of the supernatant fluid were analyzed by fluorimetric assays for dopa (*4*), 3-*O*-methyldopa (*5*), and dopamine and norepinephrine (*6*, *6a*). *S*-Adenosylmethionine was measured in portions of the supernatant fluid by a double-label, isotope dilution technique (*7*).

In agreement with previous data (*1*), administration of 500 mg of L-dopa per kilogram to 50-g male rats caused extensive polysome disaggregation in the brain 60 minutes after injection, with reduction of polysome abundance from 65 percent (controls) to 43 percent of the total ribosome profile (Table 1). Forty minutes after L-dopa injection, dopa concentrations in the brain were considerably elevated (Table 1) above the insignificant concentration found in the brains of controls. By 1 hour after injection of L-dopa, the concentration of dopa in the brain had decreased, whereas that of 3-*O*-methyldopa had doubled. This evidence that polysome disaggregation was coincident with accumulation of 3-*O*-methyldopa suggested that the *O*-methylated amino acid might be the effector metabolite. Injection of 500 mg of 3-*O*-methyldopa per kilogram resulted in considerable levels of brain 3-*O*-methyldopa at 1 hour and 2 hours (Table 1). At both of these times, concentrations of brain dopa were low but were higher than those in controls. Although the concentrations of brain 3-*O*-methyldopa were even higher than those obtained after injection of L-dopa, the polysomes were unaffected (Table 1).

Even though the product of L-dopa methylation was not the cause of polysome disaggregation, it was possible that the disaggregation resulted from the accompanying depletion of *S*-adenosylmethionine. To test this hypothesis, the effects of D-dopa and L-dopa on polysome aggregation were compared. D-Dopa also accepts a methyl group from *S*-adenosylmethionine (*3*); how-

ever, it is not converted to catecholamines (*8*). One hour after intraperitoneal injection of 500 mg of D-dopa or of L-dopa per kilogram, the concentrations of brain dopa in the two groups were similar (Table 1), and comparable brain concentrations of 3-*O*-methyldopa were formed, accompanied by similar depletions of *S*-adenosylmethionine. On the other hand, D-dopa failed to raise dopamine and norepinephrine concentrations (*8*), whereas L-dopa elevated brain dopamine concentrations to four times those of the controls and raised norepinephrine slightly. D-Dopa failed to disaggregate brain polysomes (Table 1), which implies that the formation of catecholamine metabolites from dopa is prerequisite to disaggregation.

The conversion of L-dopa to dopamine was blocked by administration of large doses (800 mg/kg) of RO4-4602. In rats treated only with this inhibitor, brain dopa concentrations rose (Table 1), thus confirming that the decarboxylase was actually inhibited. When the rats were given 500 mg of L-dopa per kilogram 30 minutes after the decarboxylase inhibitor, brain dopa attained concentrations four times greater than those obtained with L-dopa treatment alone (Table 1). In accordance with the known action of the inhibitor, this extensive increase in brain dopa was accompanied by a decrease in the concentrations of dopamine and norepinephrine to below normal values. In the presence of the decarboxylase inhibitor, L-dopa also failed to disaggregate the brain polysomes, confirming the hypothesis that a high brain concentration of L-dopa is not by itself directly responsible for polysome disaggregation.

Thus either dopamine or one of its metabolites must be responsible for the phenomenon. Norepinephrine is a less likely candidate, since only relatively few neurons can transform exogenous dopa to this catecholamine. Moreover, the effect of L-dopa on brain norepinephrine is slight (Table 1). In order to prove that the subsequent metabolism of dopamine and norepinephrine to deaminated products is not responsible for the polysome disaggregation, pheniprazine, an inhibitor of monoamine oxidase, was administered prior to L-dopa. The inhibitor alone caused the concentrations of dopamine and norepinephrine to rise (see Table 1). When L-dopa was given in graded doses 2 hours after the inhibitor, the concentrations of dopamine achieved with small

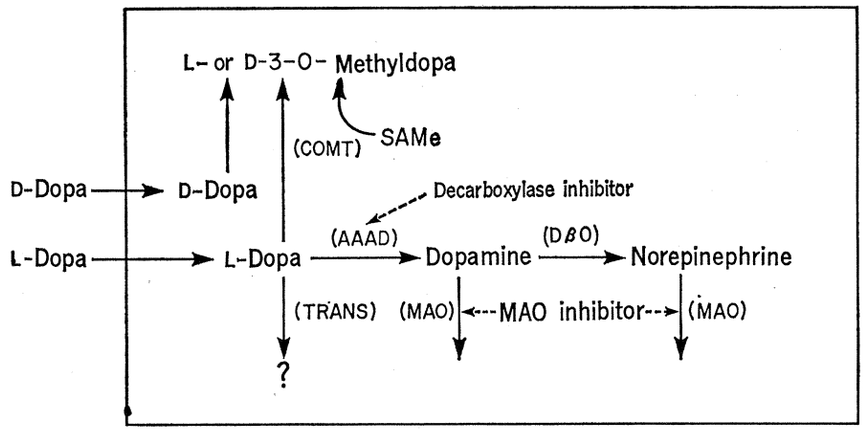

Fig. 1. Metabolism of L-dopa and D-dopa in rat brain. *COMT*, catechol-*O*-methyltransferase; *AAAD*, aromatic L-amino acid decarboxylase; *DβO*, dopamine-β-oxidase; *MAO*, monoamine oxidase; *TRANS*, dopa transaminase; and *SAMe*, *S*-adenosylmethionine. The decarboxylase inhibitor was RO4-4602; the monoamine oxidase inhibitor was pheniprazine.

doses of L-dopa (50 to 100 mg/kg) equaled those induced by much larger doses (for example, 500 mg/kg) in rats not treated with the inhibitor. This accumulation of dopamine at a low dose of L-dopa following treatment with pheniprazine was accompanied by extensive polysome disaggregation, a phenomenon not obtained when the same dose of L-dopa was given to animals that did not receive the enzyme inhibitor (Table 1).

This correlation of increased concentrations of dopamine and norepinephrine with polysome disaggregation prompted us to apply these compounds directly to the brain, since neither readily crosses the blood-brain barrier. Fifteen minutes after injecting 100 μg of dopamine intracisternally, rats had brain dopamine concentrations at least as high as those after intraperitoneal injection of L-dopa (Table 1). By 45 minutes after intracisternal treatment, dopamine had fallen to near control values. At 15 and 45 minutes after injection of 100 μg of norepinephrine, brain norepinephrine concentrations remained very high. Rats receiving either catecholamine intracisternally failed to exhibit disaggregated polysomes (Table 1) (*9*).

The failure of intracisternal administration of catecholamines to cause polysome disaggregation does not necessarily exclude these compounds as effector substances: The cerebrospinal fluid route may distribute the active agent only to superficial brain cells, leaving the major cell population unexposed (*10*); alternatively, the catecholamines placed in the cisterna magna may be so effectively concentrated within catecholaminergic neurons as to be unavailable to the other cells in the brain (*11*). Another of our studies (*12*) indicates that the accumulation of brain dopamine after administration of L-dopa is not significantly decreased in rats whose central catecholamine-containing neurons have been largely destroyed by prior intracisternal administration of 6-hydroxydopamine; hence dopamine probably is formed from exogenous L-dopa in all brain cells, even glia. It seems likely that, for the extensive polysome disaggregation to occur after the administration of L-dopa, the majority of brain cells, neurons and glia, must participate. In this connection, catecholamines have recently been shown to influence adenyl cyclase activity in glia (*13*).

Disaggregation of polysomes is usually evidence of disturbed protein synthesis; however, it remains to be demonstrated that the L-dopa–induced disaggregation described here and earlier (*1*) is actually associated with changed synthesis of brain proteins. The ability of catecholamines formed intracerebrally from exogenous L-dopa to disaggregate brain polysomes raises the possibility that catecholamine neurotransmitters released at synapses may also exert some of their effects by modifying brain polysome function.

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16 May 1972

Neuroanatomical Correlates of Morphine Dependence

Abstract. *Naloxone hydrochloride, an opioid antagonist, was applied to several discrete brain regions of morphine-dependent rats to precipitate abstinence. Severe withdrawal signs were elicited after administration in the thalamus but not in neocortical, hippocampal, hypothalamic, or tegmental areas of the brain.*

Physical dependence on morphine is manifested by a highly characteristic behavior when morphine intake is abruptly terminated or when a morphine antagonist is administered (*1*). The neuroanatomical areas related to the abstinence syndrome have not been clearly defined. Kerr and Pozuelo (*2*) reported that withdrawal signs, precipitated by opioid antagonists in the morphine-dependent rat, were suppressed or reduced when a major part of the ventromedial nucleus of the hypothalamus had been lesioned. Herz and his associates (*3*) postulated that structures in the caudal brainstem, most probably in the floor of the fourth ventricle, are important substrates for the development of dependence on morphine. In experiments in which crystalline naloxone hydrochloride was applied to discrete brain areas of the morphine-dependent rat, we find that the thalamus is one of the, if not the most sensitive, regions for precipitating withdrawal.

Male Sprague-Dawley rats (180 to 250 g) were used throughout these experiments. A 20-gauge stainless steel guide cannula, filed to a predetermined length, was stereotaxically implanted into the left hemisphere of the rat brain. Ether anesthesia was used for surgery. From 1 to 5 days after implantation of the guide cannula, dependence on morphine was induced by the subcutaneous implantation of a morphine pellet (*4*). To precipitate withdrawal, naloxone hydrochloride was applied to the brain 70 to 76 hours after pellet implantation. An inner cannula, 0.5 mm longer than the guide cannula, was tamped in crystalline naloxone hydrochloride and inserted into the guide cannula. This procedure was repeated twice to ensure that a sufficient amount of naloxone contacted the brain tissue. The total amount of naloxone hydrochloride inserted into the guide cannula ranged from 0.04 to 0.2 mg. While the precise amount of naloxone delivered to brain tissue cannot be ascertained, the treatment was uniformly administered to all animals, and the procedure successfully discriminated between brain areas of relative sensitivity. The methods and the problems associated with the application of chemicals to brain tissue have been discussed (*5*).

The abstinence syndrome precipitated by application of naloxone to the brain is similar to that observed after systemic administration of naloxone in morphine-dependent rats and will be described elsewhere (*6*). In brief, abstinence signs which are dose-dependent on naloxone, such as diarrhea, ear blanching, abnormal posturing, ptosis, teeth chattering, escape attempts, and wet shakes (*7*), appear within 10 minutes after cerebral application of naloxone in sensitive areas. Other abstinence signs such as seminal emissions and chromodacryorrhea may also be observed. Only salivation and licking movements during abstinence appear less intense when the cerebral, rather than the systemic, route of naloxone administration is utilized. Under identical experimental conditions, cerebral application of naloxone hydrochloride to the thalamus of six nondependent rats or repeated insertions of empty cannulas into morphine-dependent rats did not induce the abstinence syndrome.

The precipitated abstinence syndrome, as described above, was observed under standardized procedures. Rats were weighed and placed in 1-gallon mayonnaise jars, and naloxone hydrochloride was applied after a 10- to 15-minute adjustment period. Leaping attempts to escape from the jar and wet shakes are distinctive abstinence behavior and reflect a high degree of physical dependence. If a rat made two or more escape attempts or had three or more wet shakes within 10 minutes after cerebral application of naloxone it was considered to have undergone precipitated withdrawal and classified as exhibiting severe abstinence. This classification is based on experiments (*6*) which show that the median effective doses of naloxone for precipitating wet shakes and escape attempts are approximately five times greater than for other abstinence signs such as ear blanching, diarrhea, ptosis, swallowing movements, and teeth chattering. The anatomical correlates of

Table 1. Areas of precipitated abstinence in morphine-dependent rats.

Anatomical areas	Number in Fig. 1	Medial-lateral extension of area (mm)	Rats (No.)	Exhibiting severe abstinence (No.)
Neocortex	1	0–2.0	9	1
Hippocampus	2	0–3.0	12	0
Medial thalamus	3a	0–2.5	41	29
Lateral thalamus	3b	3.0–4.2	10	0
Diencephalic-mesencephalic junctures	4	0–2.0	20	13
Mesencephalon	5	0–2.5	16	0
Hypothalamus	6	0–2.0	7	0