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COMMENTARY

CURRENT STATUS OF DOPAMINE IN THE MAMMALIAN SPINAL CORD

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In the past, dopamine (DA) in the spinal cord was thought to be present solely in noradrenergic terminals as a precursor for the synthesis of norepinephrine (NE) [1, 2]. However, recent evidence [3–7] indicates that separate dopaminergic neurons exist in the spinal cord, and that spinal DA may serve a neurotransmitter role [3, 6, 8]. This evidence is biochemical [3, 4], pharmacological [5, 9], and electrophysiological [6]. The demonstration that there is an independent dopaminergic system in the cord, despite the relatively low steady state concentration of DA, may prove to be of more general significance. It emphasizes the possible functional importance of other amines present in the central nervous system (CNS) in low concentrations.

In the early 1960s, most authors using fluorometric methods reported that the concentration of DA in the cord was low (about 10 ng/g) [10–12]. The fluorometric methods then used to assay spinal cord DA were employed toward the limits of their sensitivity. In some cases, DA could not even be measured directly, and its value was determined as the difference between total catecholamine and NE [10]. When it became possible to visualize catecholamines in the cord with the fluorescent histochemical technique [13], it was generally assumed that all of the catecholamine fluorescence in the cord was due to NE [14]. This assumption was based, in part, on the previously published reports of low spinal DA concentration. The fluorescent histochemical technique cannot differentiate, however, between DA and NE *in situ* on a routine experimental basis. The fluorophores formed from DA and NE both have excitation and emission maxima at 410 and 480 nm respectively [15]. Subsequently, an impressive number of physiological studies were reported in which L-DOPA, the precursor of both DA and NE, was administered to increase the concentration of catecholamines in the cord. L-DOPA treatment resulted in potent and specific effects on neuronal elements associated with the monosynaptic reflex [16], the tonic stretch reflex [17] and the flexor reflex [18]. Interestingly, the effect of L-DOPA on the monosynaptic reflex in the cord was specifically blocked by pimozide [16], a DA receptor antagonist, and L-DOPA was inactive when given after a decarboxylase inhibitor [2]. Moreover, L-DOPA given in combination with a dopamine-β-hydroxylase inhibitor was shown to have the same effect on the cord as L-DOPA alone [19]. Therefore, the effects of L-DOPA must have been mediated by DA, synthesized from L-DOPA, and not by NE as has been suggested [17, 18].

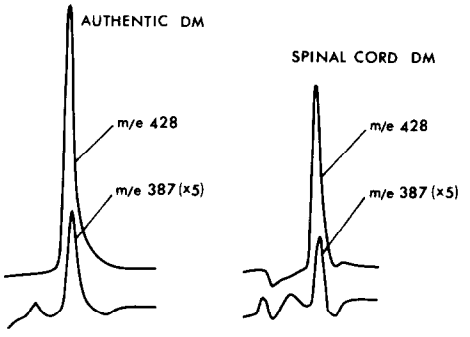
Much of the data cited already can be interpreted as support for the presence of separate dopaminergic neurons in the spinal cord. In addition, the following observations also support the idea that there are dopaminergic neurons in the spinal cord. First, the concentration of DA in the cord is substantially higher than has been reported earlier (Table 1). Second, it is doubtful whether there is any increase in the synthesis and turnover rate of NE in the central nervous system after L-DOPA administration [24–26]. Therefore, the likely candidate to mediate the effects of L-DOPA on the cord is DA and not NE. Third, apomorphine, a dopaminergic agonist, mimics the effect of L-DOPA on the flexor reflex elicited in the spinal cat [18]. Fourth, 3,4-dihydroxyphenylserine, a direct precursor of NE, does not mimic the initial effect of L-DOPA on the stretch reflex in the decerebrate rat [8].

We now present the results of recent studies that, taken together with the older literature, provide firm evidence for the existence of dopaminergic neurons in the cord. We investigated whether dopaminergic neurons could be differentiated from noradrenergic neurons in the cord by pharmacological procedures. For these studies, a gas chromatographic–mass spectrometric (g.c.–m.s.) method was used. This is the most specific and sensitive method available for identifying and quantitating catecholamines and their metabolites [27, 28]. The technique is capable of identifying and measuring less than 1 femtomole (10^{-15} mole) of DA. The DA molecule is converted to its pentafluropropionyl (PFP-DA) derivative and separated on a gas chromatographic column from other compounds. Then

Table 1. Old and new values for DA in the spinal cord

Species	Old values (ng/g)	Ref. No.	New values (ng/g)	Ref. No.
Rat	20	11	130	20
Cat	10	10	120	21
Dog	8	10	166	22
Monkey	NA*		247	22
Man	NA		180	23

* NA: not available.



	AUTHENTIC DM	BIOLOGICAL DM
1. GC RETENTION TIME	2.76 min	2.76 min
2. MID 428/387	2.62 ± 0.02 (5)	2.45 ± 0.06 (13)

Fig. 1. Identification of dopamine in the spinal cord by gas chromatography–mass fragmentography. Identical g.c. retention times and fragment ratios (428/387) for authentic and putative DA are the two parameters which make this technique specific.

PFP-DA is fragmented by electron impact, and a spectrum of the positively charged ions is obtained. Characteristic fragments having fixed mass to charge (*m/e*) values are selected from the spectrum. Subsequently, single ion monitoring is done. For DA the fragments monitored were *m/e* 428 and 387. Quantitation is achieved by adding deuterated DA as an internal standard. The specificity is based on the g.c. retention time and the values of ion fragment ratios under standard experimental conditions. These values must be statistically identical for authentic DA and putative biological DA. We know from experience that fragment ratios remain constant over a 10^4 concentration range. A typical set of results for the spinal cord is shown in Fig. 1. The conditions imposed [29] for the identification of biological DA are clearly met. We, therefore, feel confident that the substance we have measured in the cord is DA. All other compounds analyzed in this laboratory and mentioned in this study have been treated similarly.

The evidence in support of the hypothesis that there are dopaminergic neurons in the spinal cord will be discussed in sections.

Selective depletion of DA in the spinal cord

If DA in the cord is present in noradrenergic neurons, then DA should decrease in parallel with the decrease of NE when noradrenergic neurons are destroyed. This does not occur. Benztropine plus 6-hydroxydopamine (6-HDA) causes a significant depletion of NE, yet DA levels are unaffected. Conversely, treatment with desipramine (DMI) plus 6-HDA causes a significant selective depletion of DA in the cord; the levels of NE are unaffected. DMI and benztropine act as relatively specific inhibitors of the uptake of 6-HDA into noradrenergic and dopaminergic neurons, respectively, thereby protecting them from destruction by the neurotoxin (see Table 3 of Ref. 5). This pharmacological result suggests that the bulk of the DA in the cord is not present in noradrenergic neurons.

A result similar to the above has been demonstrated using an entirely different technique. The locus coeruleus (LC) provides a purely noradrenergic innervation to the spinal cord [30, 31]. When this noradrenergic innervation was destroyed by a bilateral electrothermic lesion of LC, the NE levels were reduced 40–70 per cent in different regions of the cord. The levels of DA were not affected by this operation (Table 2). This result again indicates clearly that most of the DA in the cord is not localized in noradrenergic neurons.

A further demonstration of the pharmacological separation of dopaminergic and noradrenergic neurons comes from experiments in which 6-HDA was injected into the left substantia nigra. This caused a significant depletion of DA on the left side of the cord. The concentration of DA on the right side was unaffected, while the NE concentration was not affected on either side (Table 3).

Lastly, Magnusson [4] found that a mid-thoracic cordotomy resulted in decay of DA and NE in the cord

Table 2. Levels of dopamine and norepinephrine in various regions of the spinal cord after bilateral lesion of the locus coeruleus*

	Norepinephrine (nmoles/g)		Dopamine (nmoles/g)	
	Control	Lesioned	Control	Lesioned
Dorsal horn				
Cervical	4.7 ± 0.1 (6)	2.8 ± 0.3† (9)	0.56 ± 0.03 (6)	0.48 ± 0.04 (9)
Thoracic	3.6 ± 0.2 (6)	2.8 ± 0.4‡ (9)	0.42 ± 0.04 (6)	0.48 ± 0.06 (9)
Lumbar	3.8 ± 0.2 (6)	2.3 ± 0.2† (9)	0.33 ± 0.02 (6)	0.31 ± 0.02 (9)
Ventral horn				
Cervical	4.0 ± 0.4 (6)	1.6 ± 0.3† (9)	0.22 ± 0.03 (6)	0.18 ± 0.04 (9)
Thoracic	3.9 ± 0.2 (6)	1.6 ± 0.3† (9)	0.07 ± 0.03 (6)	0.09 ± 0.06 (9)
Lumbar	3.9 ± 0.2 (6)	1.6 ± 0.2† (9)	0.13 ± 0.02 (6)	0.12 ± 0.03 (9)
Zona intermedia				
Cervical				
Thoracic	4.4 ± 0.2 (6)	1.9 ± 0.3† (9)	0.29 ± 0.04 (6)	0.22 ± 0.05 (9)
Lumbar				

* The lesioning electrode was stereotaxically placed in the locus coeruleus. A direct current of 2.5 mA was passed for 45 sec. Ten days later the spinal cord was analyzed for DA and NE.
† $P < 0.001$.
‡ $P < 0.01$.

Table 3. Depletion of DA in the left thoracic cord by 6-HDA injected into the left substantia nigra*

	Left		Right	
	DA	NE	DA	NE
	nmoles/g ± S.E.M. (N = 4)			
Control	0.29 ± 0.005	2.1 ± 0.2	0.32 ± 0.03	2.1 ± 0.1
6-HDA	0.14 ± 0.02†	1.9 ± 0.1	0.31 ± 0.03	1.8 ± 0.1

* Four μg 6-HDA in 2 μl solvent was stereotaxically placed into the left substantia nigra. Ten days later the cord was removed and the thoracic region was analyzed for DA and NE.

† $P < 0.05$, when compared with control.

caudal to the cut at entirely different rates. Based on this result, he has argued in favor of the existence of a spinal dopaminergic neuronal system.

Metabolism of DA in the cord

Using the monkey as their experimental animal, Kessler *et al.* [3] blocked the flow of cerebrospinal fluid from the brain. Then they perfused the subarachnoid space of the lumbar cord, and measured homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG), major metabolites of DA and NE, respectively, in the perfusate. The rates of production of HVA and MOPEG were 2.4 and 1.4 ng/ml respectively. These results suggest that DA in the cord is metabolized almost twice as fast as NE, and, therefore, the utilization of DA in the cord must be much higher than that of NE. Apparently, the functional importance of DA in the cord is much greater than is suggested by its relatively low concentration, compared with NE.

Some neuroleptic drugs, known to block the DA receptor in the central nervous system [32] have been shown to increase DA metabolism in the cord (see Table 5 of Ref. 5). Blockade of the DA receptor by these agents in the brain has been shown to lead to an activation of the dopaminergic neuron [33], possibly by activation of a negative feedback mechanism. The net result is an increased release and metabolism of DA. This is measured as an increase in the metabolites of DA. We have investigated the effects of three neuroleptics on DA metabolism in the spinal cord: chlorpromazine, clozapine and haloperidol. All three increased DA metabolism in the striatum, as expected. Chlorpromazine and clozapine increased DA metabolism in the cord to the same extent as they did in the striatum (see Table 5 of Ref. 5). Haloperidol was ineffective in the cord at the single time interval and dose studied. A more detailed investigation is now required to determine whether haloperidol does, indeed, block the dopamine receptor in the spinal cord, or whether the effect was missed at the time interval studied. The result, however, suggests that there are DA receptors in the cord and that DA metabolism in the cord might be regulated by neuronal feedback loops. The HVA measured in the cord did not originate in the brain. If it had, the apparent increase of HVA in the cord would have occurred after haloperidol treatment as well.

A third method by which DA metabolism in the cord was increased was by electrical stimulation of the left substantia nigra. This caused a highly significant increase of HVA on the left side of the thoracic cord. There was no corresponding increase on the right side (Table 4). This result suggests that there is a nigrospinal dopaminergic projection to the thoracic cord and that the fibers are uncrossed.

Electrophysiological data

Using the iontophoretic technique, the effects of DA,

Table 4. Effect of electrical stimulation of left substantia nigra on the metabolism of dopamine in the thoracic spinal cord*

	DA	HVA
	nmoles/g ± S.E.M. (N)	
Unanesthetized control		
LS	0.34 ± 0.04 (5)	0.25 ± 0.03 (4)
RS	0.35 ± 0.04 (3)	0.26 ± 0.03 (4)
Anesthetized control		
LS	0.43 ± 0.06 (5)	0.32 ± 0.03 (5)
RS	0.43 ± 0.03 (5)	0.31 ± 0.04 (5)
Anesthetized stimulated		
LS	0.51 ± 0.06 (4)	0.82 ± 0.23† (5)
RS	0.50 ± 0.04 (4)	0.25 ± 0.02 (5)

* The stimulating parameters were: 15 Hz, 400 μA, and 2.0 msec for 1 hr. The spinal cord was removed immediately afterward and the thoracic region was analyzed for DA and HVA.

† $P < 0.01$, when compared with the anesthetized control.

NE and 5-hydroxytryptamine (5-HT) on the excitability of motoneurons were assessed [6]. The effect of DA on the motoneuron field potential was blocked by α-flupenthixol, a specific dopaminergic antagonist. α-Flupenthixol did not antagonize the effect of either NE or 5-HT on the motoneurons. Apparently, DA receptors are present in the cord, and they are involved in controlling the excitability of motoneurons.

Differential distribution of DA in the cord

The results in Table 2 show that DA is distributed differentially in the cord. The concentration of DA in the dorsal horn is two to three times higher than that in the ventral horn. The NE:DA ratio is 8:1 in the cervical dorsal region of the cord; in the cervical ventral horn the ratio is 18:1. However, the concentrations of NE in both the cervical dorsal and ventral horns are almost the same. This contrasts with some peripheral tissues and the cerebellum, which are thought to have a purely noradrenergic innervation [5]. The NE:DA ratio in these tissues is 50:1. Therefore, in a noradrenergic neuron DA appears to represent about 2 per cent of the total cell catechoamines. Apparently most of the DA in the cord, with the possible exception of the ventral horn of the thoracic region, is non-precursor DA. Other authors, basing their argument on different criteria, support this conclusion [4, 7].

Synthesis of DA and NE in the cord

The rates of synthesis of DA and NE in the cord are 0.85 and 0.17 nmole/g/hr respectively [4]. DA, therefore, is synthesized at a rate that is about five times greater than is necessary to maintain the synthesis of NE. Presumably about 80 per cent of DA in the cord is non-precursor DA [4].

Spinal DA present in terminals of descending fibers

Several days after a mid-thoracic cordotomy, DA caudal to the cut is reduced significantly. Rostral to the cut, DA levels are unchanged (see Table 6 of Ref. 5). DA is present apparently in the terminals of descending fibers, and not in interneurons. From the previous discussion, it appears that some of the cell bodies of these descending fibers are localized in the substantia nigra.

Other indirect evidence for the existence of dopaminergic neurons

Reserpine induces a dose-dependent depletion of DA in the cord (see Fig. 1 of Ref. 5). Reserpine apparently impedes the transport of amines into storage vesicles thus exposing them to degradative enzymes in the cytosol [34]. Reserpine does not inhibit dopa decarboxylase [35]. Therefore, if DA were normally present only in the cytosol as a precursor of NE, then reserpine should not reduce DA stores significantly. This result suggests that DA in the cord, like other amines in the CNS, is probably normally sequestered in a vesicular store.

GENERAL COMMENTS

The results we have discussed strongly support the hypothesis that there are dopaminergic neurons in the cord and that DA serves as a neurotransmitter in the cord. Two important experiments, however, remain to be done. One is to demonstrate directly that DA is released in the cord, and to show that the release is stimulus dependent. The second is to demonstrate that the DA released by stimulation has the same effect on a neuronal element as DA iontophoretically applied to that same element. The common effect of the endogenous and exogenous DA should then be antagonized by a specific dopaminergic antagonist. The evidence already available suggests very strongly that the results of these experiments will be positive.

The pattern of distribution of DA in the cord implicates it in a variety of physiological functions. The highest concentration is found in the dorsal horn of the cervical, thoracic and lumbar regions. DA, therefore, may be involved in the processing of sensory information. The neurophysiological data already available [17] provides strong indirect evidence that in the ventral horn of the lumbar cord DA is involved in regulating the level of activity of gamma motoneurons, and hence in controlling the sensitivity of the stretch reflex loop. Other authors [17–19] have attributed this effect previously to NE, presumed to be formed after the administration of L-DOPA. However, a recent study has failed to show any significant increase in the rate of synthesis of NE in the CNS after L-DOPA infusion [24]. This result and others [25, 26] cast doubt on the often stated conclusion that the mechanism of action of L-DOPA on spinal transmission is due to the increased synthesis and release of NE. In the zona intermedia, the area of origin of the preganglionic sympathetic neurons, the concentration of DA is also relatively high, implicating DA in the regulation of sympathetic function as well.

There are two pathological situations in which spinal cord DA may be important: spinal trauma and Parkinson's disease. The DA [21, 36, 37] but not the NE [38] concentration is elevated in the cord after experimental spinal trauma. The neurological deficits associated with spinal trauma might be ameliorated by drugs that interfere with the action of DA. Specific dopaminergic blockers should be used as the first drugs of choice to test this hypothesis. Other agents, like catecholamine synthetic enzyme inhibitors, are likely to compromise the noradrenergic system as well, and the relative importance of DA and/or NE in mediating the neurological effects of spinal trauma will still be left unresolved [38].

Are DA levels reduced in the spinal cord, as they are in the striatum, in Parkinsonian patients? DA levels in the normal humal spinal cord, obtained within 4 hr of death, are approximately equal to the levels found in animals [23]. If DA is reduced in the cord in these patients, this finding will raise the possibility that both the manifestations of the disease (akinesia, rigidity and tremor) and the beneficial and/or adverse effects (improved muscular tone and involuntary movements and postural hypotension) [39] of L-DOPA therapy could involve spinal mechanisms directly. The motoneurons are the final common pathway from the CNS to muscle, and Parkinsonism is essentially a disease of motor deficits. Indirect neurophysiological evidence from animal studies certainly suggests that DA affects the functioning of motoneurons [6, 16–19]. A better understanding of the disease process, the site of action of L-DOPA, and the mechanisms of action of L-DOPA could be beneficial for developing new therapeutic agents.

In this commentary we have attempted to draw attention to a relatively neglected, but potentially important aspect of current research in monoamines in the CNS, that is, the evidence in favor of there being a dopaminergic system in the spinal cord. The normal and pathophysiological implications of such a system could be quite important. We hope that much more attention will be directed to this area as a result of this commentary. It should also be clear that low levels of a putative neurotransmitter in a tissue cannot be a sufficient reason for dismissing it as being functionally unimportant in that tissue. It could be that the few nerve terminals in that tissue provide a specific innervation to a relatively small population of neurons. The specific activity of the putative neurotransmitter within the restricted area of its functional importance, therefore, could be quite high. As proof of this, there is now good evidence that there are adrenaline-containing neurons in the spinal cord [40–42]. The spinal concentration of adrenaline is even lower than the concentration of DA [42]. Clearly, adrenaline in the cord is not a precursor. Instead, all the adrenaline terminals seem to be restricted to the zona intermedia, especially the lateral column nucleus [41]. This suggests that adrenaline in the thoracic cord is concerned specifically with the regulation of preganglionic sympathetic neurons and not, for example, with the regulation of somatic motor neurons. In the future, it might be necessary to define more precisely the area or nucleus of the CNS which has been studied. One should also be aware of the inadequacy of reporting transmitter concentration as a unit weight per g of tissue or mg of protein. Ideally, one should have some idea of the number of neurons being studied. The problems are bound to come up again, as analytical procedures become more powerful and refined.

The observations concerning the possible functional importance of low concentrations of putative neurotransmitters in the cord raise important general questions for the study of low levels of putative neurotransmitters elsewhere in the CNS. This subject has been addressed recently [43]. It might be that many of these so-called trace amines are functionally unimportant. On the other hand, they could be pointing us toward a new era of our understanding of the chemical organization of the CNS.

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