

## Prodynorphin-derived Peptide Expression in Primate Cortex and Striatum

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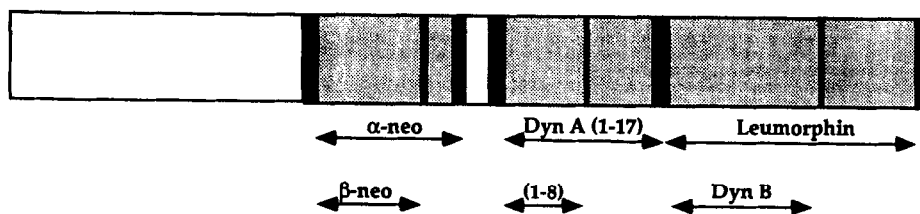
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**Abstract**—The distributions of four prodynorphin-derived peptides, dynorphin A (1-17), dynorphin A (1-8), dynorphin B, and  $\alpha$ -neo-endorphin were determined in 10 cortical regions and the striatum of the old world monkey (*Macaca nemestrina*).  $\alpha$ -neo-endorphin was the most abundant peptide in both cortex and striatum. The concentrations of all four peptides were significantly greater in the striatum compared to the cortex. In general, concentrations of each peptide tended to be higher in allocortex than in neocortex. Possible inter- and intradomain processing differences, as estimated by ratios of these peptides, did not vary within cortex, but the intradomain peptide ratio, dyn A (1-17)/dyn A (1-8), was significantly greater in cortex than in striatum. These results indicate that prodynorphin is, in some ways, uniquely processed in the primate. Particularly unusual is the relatively low abundance of prodynorphin-derived products in the cortex, in the face of moderately high levels of kappa opiate receptor expression.

### Introduction

Prodynorphin, proenkephalin, and pro-opiomelanocortin are the 3 propeptides that serve as precursors to endogenous opioid peptides. These 3 precursors make a number of peptide ligands with affinities at opiate receptors. Prodynorphin produces ligands with varying affinities for the  $\mu$  and  $\delta$  opiate receptors,<sup>9</sup> but differs from pro-opiomelanocortin and proenkephalin by also producing peptides with affinity for the  $\kappa$  opiate receptor.<sup>1</sup> 3 domains have been identified in the prodynorphin molecule, the dynorphin A, leu-

morphin, and neoendorphin domains.<sup>5</sup> Dynorphin A, which is dynorphin A (1-17) (dyn A (1-17)), can be further processed into dynorphin A (1-8) (dyn A (1-8)) (Fig. 1).<sup>14,17</sup> Dyn A (1-17) has greater affinity for the  $\kappa$  opiate receptor than dyn A (1-8).<sup>9</sup> The leuomorphin domain can be further processed into dynorphin B (dyn B).<sup>15</sup> Dyn B has an affinity for the  $\kappa$  opiate receptor similar to dyn A (1-17).<sup>9</sup> The neoendorphin domain, which is  $\alpha$ -neo-endorphin ( $\alpha$ -neo) can be further processed into  $\beta$ -neo-endorphin ( $\beta$ -neo).<sup>6,13</sup>  $\alpha$ -neo has an affinity for the  $\kappa$  opiate receptor similar to dyn B and dyn A (1-17).<sup>9</sup> These 3 molecules also bind to  $\mu$  and  $\delta$  opiate receptors, but these affinities are 6 to 20 times less than their affinities for the  $\kappa$  opiate receptor.<sup>9</sup> The extent of how each domain is processed may thus



**Fig. 1** Schematic representation of the prodynorphin molecule. There are 3 domains, the neoeendorphin domain ( $\alpha$ -neoeendorphin ( $\alpha$ -neo) potentially processed into  $\beta$ -neoeendorphin ( $\beta$ -neo)), the dynorphin A (dyn A) domain (dyn A (1-17) potentially processed into dyn A (1-8)), and the leumorphin domain potentially processed into dynorphin B (dyn B)).

alter the functional opioid selectivity of the domain.<sup>2</sup> However, the physiological activity of prodyn is not only determined by expression and processing of these domains, but also by the specific anatomical locations of where the peptides (and associated opiate receptors) are expressed.

Prodynorphin has been most extensively studied in the rodent. A specific pattern of distribution of prodynorphin-containing cells and fibers in rodent brain has been found using immunocytochemistry, with prodynorphin being visualized in the caudate-putamen, substantia nigra, hippocampus, hypothalamus, amygdala, parabrachial nucleus, nucleus tractus solitarius, substantia grisea centralis, as well as in much of the cortex.<sup>4,8,12,17,22</sup> Kappa receptors have been visualized via receptor autoradiography, with their distribution being similar to that of the prodynorphin-containing fibers.<sup>11,12</sup> This would be expected, given that prodynorphin is the only known source of endogenous kappa ligands. Prodynorphin derived peptide concentrations have also been examined in dissected brain regions, using radioimmunoassay. In general, the distribution of immunoreactive prodynorphin derived peptides

parallels the distribution of both prodynorphin fibers and  $\kappa$  receptor binding in rodent brain. Further, concentrations of various prodynorphin derived peptides appear to be similar in the rodent striatum and cortex.<sup>17</sup> In those regions in which fairly detailed examination of the various prodynorphin derived peptides have been performed (primarily rat striatum and cortex), several consistent observations have been made. In the rat brain,  $\alpha$ -neo is, by far, the most abundant peptide present. The A and B domains are present in much lower concentrations, and the A domain is preferentially processed to dyn A (1-8).

The primate prodynorphin/kappa system has not been studied as extensively as in the rat. The distribution of prodynorphin-containing cells and fibers is similar in the primate, although the density of these cells is greater in primate cortex than in rodent cortex.<sup>3,7,10</sup> Interestingly, the density of kappa receptors in the cortex also seems to increase with phylogeny, with cortical kappa receptors being far more abundant in the primate than the rodent.<sup>10,19</sup> Detailed examination of the specific forms of prodynorphin-derived peptides expressed in primate brain, however, has not been performed.

We undertook this study to begin to characterize the expression of specific forms of prodynorphin in selected brain regions of the primate. Given the relative abundance of prodynorphin fibers and cells, as well as  $\kappa$  receptors in the cortex of the primate compared to the rodent, we have concentrated on the examination of the cortex, comparing it to the striatum. We have therefore determined the concentrations of 4 prodynorphin-derived peptides, representing all three functional domains of the propeptide, in neocortical, allocortical, and striatal regions of the old-world monkey brain.

**Table 1** Cortical regions studied

Region studied	Corresponding cortical areas <sup>21</sup>	Function or common name
O	OB/OC	visual
TA	TA	primary auditory
PG	PG	secondary association (IPL)
FD	FD	prefrontal
L	LA, LC	cingulate
FA	FA	motor
A	A	temporal allocortex
PB	PB, PC	primary somatosensory
FB	FB	secondary motor (premotor)
PE	PE	secondary somatosensory (area 5)

## Methods

### Animals

The brains of 6 old world monkeys (*Macaca nemestrina*) were obtained from several sources. All monkeys were in good health and medication-free at the time of tissue acquisition. These monkeys were all sacrificed for other studies, and the brains were rapidly removed and frozen at  $-80^{\circ}\text{C}$  for later study.

### Tissue preparation

Frozen whole brains were removed from  $-80^{\circ}$  storage and kept on wet ice until sufficiently thawed to dissect (approximately  $-20^{\circ}$ ). Brains were cut into 5–10 mm slabs in a coronal plane. These slabs were conservatively dissected on wet ice into the specific regions of interest, using the atlas and nomenclature of Von Bonin and Bailey.<sup>21</sup> Dissected tissue was promptly frozen on dry ice and stored at  $-80^{\circ}\text{C}$  until extracted for assay. The individual regions were weighed while frozen, and immediately homogenized in GT buffer (10 mM EDTA, 50 mM Tris pH 7.5, and 5M guanidine thiocyanate) with a Brinkman Polytron. 6 volumes of 4M LiCl were added to each sample, and samples were placed on ice overnight. The samples were centrifuged for 30 sec at 10000 rpm and the supernatant was transferred to a clean tube. Glacial acetic acid was added to a final volume of 5% and each sample was extracted using Sep-Pak C<sub>18</sub> cartridges, as previously described.<sup>17</sup> The eluant from the cartridges was dried with a Savant Speed Vac rotary evaporator. The dried extract was resuspended in methanol:0.1 N HCl (1:1, v:v), and stored at  $-20^{\circ}\text{C}$  until assayed.

### Radioimmunoassays

Radioimmunoassays (RIAs) were performed as previously described.<sup>17,20</sup> Briefly, diluted tissue sample or peptide standard, <sup>125</sup>I-labeled peptide (8000–10000 cpm), and diluted antiserum were incubated in a total volume of 300  $\mu\text{l}$  overnight at  $4^{\circ}\text{C}$ . The reaction was terminated by the addition of 0.6 ml of charcoal slurry, and the assay tube centrifuged for 10 min at  $7500 \times g$ . The pellet was discarded and the supernatant counted in a TM Analytic 1290 gamma counter. All samples were assayed in trip-

licate. Radiolabeled peptides for RIA were prepared by chloramine-T-mediated iodination.<sup>6</sup> Antisera were raised in rabbits, and were the gift of Dr Huda Akil (Mental Health Research Institute, University of Michigan).

### Data analysis

Peptide concentration values from 3 independent assays were averaged, and used for subsequent data analysis. Prodynorphin can be processed into three domains<sup>2</sup> (Fig. 1). To estimate potential variations of interdomain processing, the ratios of  $\alpha$ -neo/[dyn A (1–17) + dyn A (1–8) (hereafter, total dyn A)] and dyn B/total dyn A were calculated. These ratios would be expected to be unity if prodynorphin was processed into equimolar amounts of the three domains, though because  $\beta$ -neo and leumorphin were not assayed, these ratios might be somewhat less than unity. To estimate possible processing differences within one prodynorphin domain, the ratio dyn A (1–17)/dyn A (1–8) was calculated. The dyn A region was chosen because dyn A (1–17) and dyn A (1–8) have different affinities for the opiate receptors, and processing differences may thus be important physiologically. Potential regional differences were determined by analysis of variance, with post-hoc Dunnett's t-test.

## Results

### Peptide content

The concentrations of the 4 prodynorphin derived peptides were determined in the caudate, putamen, and 10 selected cortical regions, as shown in Figure 2 and Table 2. For each peptide, the concentrations in the caudate and putamen were quite similar, although  $\alpha$ -neo tended to be more abundant in the putamen than in the caudate.  $\alpha$ -neo was the most abundant of the 4 peptides in both striatal regions, which was 5–8 times the concentration of dyn B. The concentration of dyn A (1–8) was double that of the concentration of dyn A (1–17). The total dyn A concentrations were comparable to the concentration of dyn B for both striatal regions.

All 4 peptides were also found in the cortex, but at considerably lower concentrations (Fig. 2 and Table 2). There were no significant differences in the concentrations for each peptide between individual

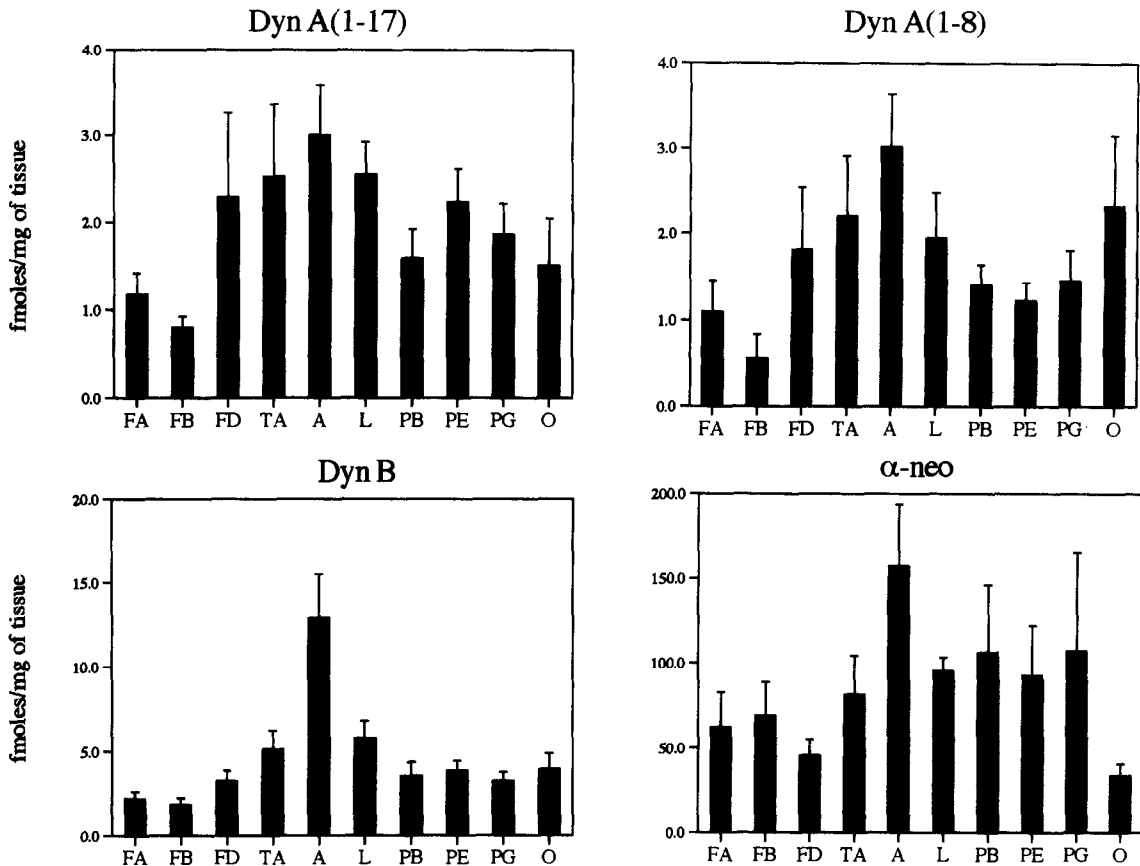


Fig. 2 Peptide concentrations in 10 cortical regions of the old world monkey, expressed as means ( $\pm$ SEM) in fmol/mg of wet tissue. There were no significant regional differences, except for dyn B, in which allocortex levels were higher than neocortical concentrations, a trend seen for the other three peptides as well. For abbreviations, see Table 1.

cortical regions (dyn A (1-17),  $F = 1.64$ ; dyn A (1-8)  $F = 1.78$ ;  $\alpha$ -neo  $F = 1.45$ ; for all,  $df = 9, 50$ ,  $p = \text{N.S.}$ ) except dyn B ( $F = 8.25$ ,  $df = 9, 50$ ,  $p < 0.0001$ ) (Fig. 2). The significance across cortical regions seen for dyn B concentration was predominantly due to higher levels in the temporal allocortex, a trend also seen for the other three

peptides, though reaching significance only for dyn B.

Mean values for concentrations of each peptide in cortex are summarized in Table 2. Because of the significant increase of dyn B and a trend toward an increase of other peptides in allocortex, the cortical regions have been separated into neocortex and

Table 2 Comparison of expression of prodynorphin-derived peptides in striatum and cortex

Peptide	Caudate	Putamen	Neocortex	Allocortex	Neocortex caudate	Allocortex caudate
dyn A (1-17)	21.80 $\pm$ 5.50	24.00 $\pm$ 16.28	1.84 $\pm$ 0.18	3.00 $\pm$ 0.58	0.08	0.14
dyn A (1-8)	49.33 $\pm$ 10.11	51.91 $\pm$ 20.79	1.56 $\pm$ 0.18	3.02 $\pm$ 0.63	0.03	0.06
dyn B	75.11 $\pm$ 5.52	75.81 $\pm$ 27.62	3.65 $\pm$ 0.28	12.88 $\pm$ 2.63	0.05	0.17
$\alpha$ -neo	397.87 $\pm$ 45.84	597.61 $\pm$ 60.79	76.89 $\pm$ 9.41	157.08 $\pm$ 36.54	0.19	0.39

Values are means  $\pm$  SEM, expressed as fmol/mg of tissue. Putamen values represent the averages of 3 animals, while the values for the other structures are the averages of 6 animals.

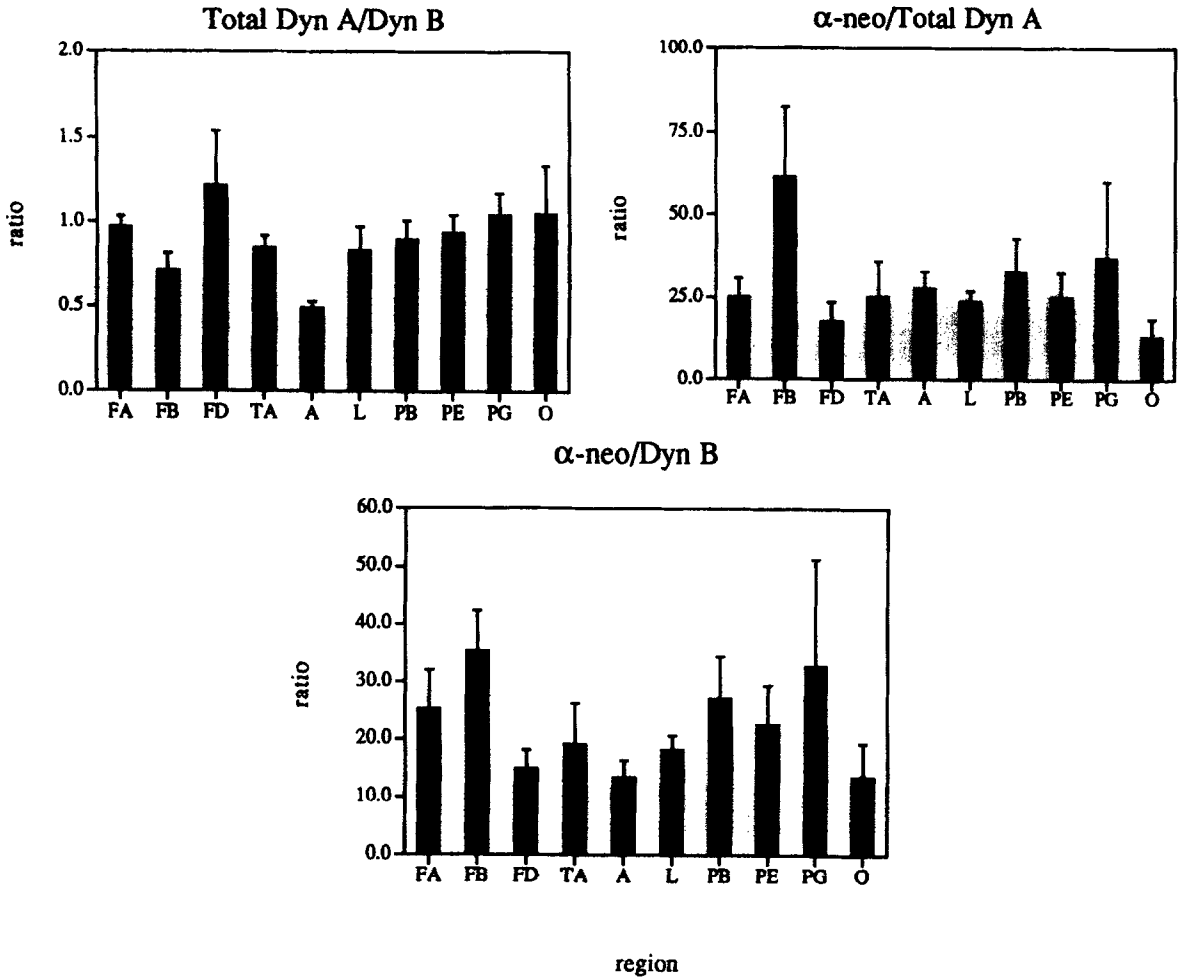


Fig. 3 Interdomain processing in 10 cortical regions, as estimated by ratios of the 3 domains of prodynorphin, expressed as ratios  $\pm$  SEM. There were no significant regional differences in the interdomain processing ratios.

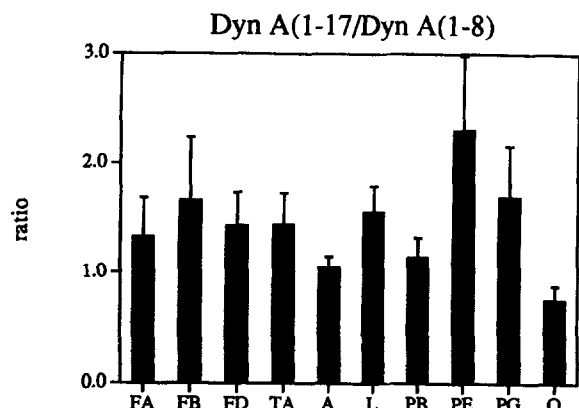
allocortex in this table. As also seen in Figure 2, levels of each peptide were 2–3.5 times higher in allocortex than in neocortex. Like the striatal regions,  $\alpha$ -neo was the most abundant peptide in the cortex, followed by dyn B. The peptides derived from the dyn A region were least abundant.

*Interdomain processing*

To estimate the extent of interdomain processing in the striatum and cortical regions, the ratios of total dyn A/dyn B,  $\alpha$ -neo/total dyn A, and  $\alpha$ -neo/dyn B were calculated. The ratio of total dyn A/dyn B was similar in the caudate and the putamen. The other 2 ratios in the putamen were twice those seen in the caudate, which was accounted for by the higher

concentration of  $\alpha$ -neo in the putamen compared to the caudate. The cortical ratios of  $\alpha$ -neo/total dyn A and  $\alpha$ -neo/dyn B tended to be higher than those in the striatum ( $\alpha$ -neo/total dyn A ( $F = 1.61$ ,  $df = 11, 57$ ,  $p = N.S.$ ) and  $\alpha$ -neo/dyn B ( $F = 1.38$ ,  $df = 11, 57$ ,  $p = N.S.$ ). The total dyn A/dyn B ratio was approximately unity in the striatum and in the cortex ( $F = 1.26$ ,  $df = 11, 57$ ,  $p = N.S.$ ) (Table 3). There were no significant regional differences in the 3 ratios found in cortex (Fig. 2).

In Table 3, the mean ratios have again been separated into neocortex and allocortex. All 3 ratios tended to be greater in the neocortex than in the allocortex, especially total dyn A/Dyn B. For the total dyn A/Dyn B ratio, the values in the neocortex



**Fig. 4** Intradomain processing in the cortex, estimated as the ratio of dyn A(1-17)/dyn A(1-8). This ratio was significantly lower in the caudate and putamen compared to the cortex, though there were no significant differences between cortical regions.

are similar to those in the striatum, while the allocortex ratio is approximately half of the neocortical and striatal ratios. For the 2  $\alpha$ -neo ratios, the neocortical values tended to be greater than the allocortical values and both tended to be greater than the corresponding striatal values.

#### *Intradomain processing*

To estimate intradomain processing of the dyn A domain in striatal and cortical regions, the ratio of dyn A (1-17)/dyn A (1-8) was calculated (Table 3). The dyn A (1-17)/dyn A (1-8) ratio was similar in the caudate and the putamen, but was significantly less than in the cortex ( $F = 2.33$ ,  $df = 11, 55$ ,  $p < 0.05$ ), with cortical values 2-3 times greater than striatal values. There were no significant differences within cortical regions ( $F = 1.35$ ,  $df = 9, 48$ ,  $p = N.S.$ ). When the neocortex and the allocortex were assessed separately, the neocortex tended to be about 1.5 times greater than the allo-

cortex. Cortical regions appear to have higher concentrations of dyn A (1-17), relative to dyn A (1-8), than does the striatum.

#### **Discussion**

Prodynorphin derived peptides have not been as extensively studied in primate brain as in rodent brain. While prodynorphin fibers and cells have been visualized in the primate brain, and are relatively abundant in the primate cortex and striatum, little is known concerning the expression of specific forms of prodynorphin derived peptides. This study has further characterized the prodynorphin system in the primate, by determining concentrations of prodynorphin derived peptides in the cortex and the striatum. Prodynorphin derived peptides were identified in all regions surveyed, but the concentrations in striatum were appreciably greater than those found in various cortical regions. Further, prodynorphin appears to be differentially processed in the striatum and cortex, especially in how the dyn A domain is handled.

As seen in previous reports concerning rodent cortex and striatum, the general pattern of expression of prodynorphin derived peptides in primate brain was  $\alpha$ -neo  $>$  dyn B  $\geq$  total dyn A. Given that we did not assay leuomorphin or  $\beta$ -neo, the predominance of  $\alpha$ -neo may be even more pronounced than we found. One hypothesis to explain this relative abundance of  $\alpha$ -neo, first suggested for the rodent prodynorphin system,<sup>20</sup> is that there are differential rates of processing of the 3 domains. Leu-enkephalin is the theoretical end product of the processing of all 3 domains. The cleavage site for dyn A (1-8) and dyn B to be further processed into leu-enkephalin is composed of arginine-argi-

**Table 3** Comparison of processing ratios of prodynorphin-derived peptides in striatum and cortex

Ratio	Caudate	Putamen	Neocortex	Allocortex	Neocortex caudate	Allocortex caudate
dyn A (1-17)/ dyn A (1-8)	0.43 $\pm$ 0.05	0.37 $\pm$ 0.12	1.47 $\pm$ 0.13	1.05 $\pm$ 0.10	3.42	2.44
dyn A/dyn B	0.93 $\pm$ 0.16	0.92 $\pm$ 0.12	0.95 $\pm$ 0.06	0.49 $\pm$ 0.04	1.02	0.53
$\alpha$ -neo/dyn A	6.30 $\pm$ 0.94	12.41 $\pm$ 4.71	29.06 $\pm$ 4.19	27.60 $\pm$ 5.29	4.61	4.38
$\alpha$ -neo/dyn B	5.25 $\pm$ 0.38	10.29 $\pm$ 3.30	23.23 $\pm$ 2.77	13.46 $\pm$ 2.92	4.42	2.56

Putamen values (ratios  $\pm$  SEM) represent the averages of 3 animals, while values for the other structures are the averages of 6 animals.

nine residues, while the  $\beta$ -neo cleavage site for subsequent processing is arginine-lysine. As has been previously suggested,<sup>20</sup> the arginine-lysine site in the neoendorphin domain may be less favorable for cleavage than the arginine-arginine sites in the other 2 domains, and thus cleavage to leu-enkephalin may be favored in the dyn A and leuomorphin domains. The end result of this would be less processing and the relative accumulation of the neoendorphin domain.

Unlike the rodent, however, the striatum of the primate had greater concentrations of all 4 peptides than the cortex. This is similar to an earlier report restricted to concentrations of dyn B in primate brain.<sup>16</sup> This is interesting, and perhaps unexpected, considering  $\kappa$  receptor binding has been reported to be greater in the cortex than in the striatum.<sup>10,16,19</sup> One may well expect an increase in the number of binding sites to be associated with a parallel increase in the concentration of ligand for that receptor, which was not observed. This could potentially be explainable with higher rates of turnover of the endogenous  $\kappa$  ligands in the primate versus the rodent, or even the existence of other endogenous high-affinity  $\kappa$  ligands. A more likely possibility is that cortical prodyn may be processed differently in the primate, with a shift toward peptides with relatively higher  $\kappa$  affinities.

While there appeared to be no heterogeneity between most regions of the primate cortex, the allocortical regions tended to have higher concentrations of the 4 prodynorphin peptides than the neocortical regions, with dyn B reaching significance. This is similar to what is observed in the rodent cortex,<sup>17</sup> and may suggest a greater role of prodynorphin in allocortical function that is evolutionarily preserved.

Several differences of peptide processing, estimated by inter- or intradomain processing ratios, were observed in the primate, compared to similar indices in the rodent. The  $\alpha$ -neo/dyn B and  $\alpha$ -neo/total dyn A interdomain ratios tended to be 3–4 times greater in primate cortex compared to striatum. While these values are somewhat similar to those observed in the rodent, these ratios are not different between rodent striatum and cortex.<sup>17</sup> Given that  $\alpha$ -neo is the most abundant form in all studied regions of primate, the relative increase in  $\alpha$ -neo in primate cortex suggests that this domain

contributes more to the  $\kappa$  tone of the cortex than the striatum in the primate. The leuomorphin and dyn A domains may be processed into leu-enkephalin at a greater rate, in primate and rodent, but the greater processing difference in the primate cortex may mean that prodynorphin is also a significant source of  $\delta$  tone in the cortex. The total dyn A/dyn B ratio is approximately unity in the primate cortex and striatum, while the ratio in the rodent has been reported to be 2–4.<sup>17,20</sup> As leuomorphin was not assayed, this ratio in primate may even be less than unity. This suggests that the dyn A domain may be handled differently in the primate compared to the rodent, and processed to leu-enkephalin preferentially over the leuomorphin and neoendorphin domains.

Intradomain processing of the dyn A domain, estimated by the ratio of dyn A (1–17)/dyn A (1–8), also reflected some interesting relationships. The primate striatum ratio value was similar to those reported in the rodent striatum and cortex, all being 0.20–0.43.<sup>17,20</sup> However, the primate neo- and allocortical ratios were greater than unity. Even though the absolute content of dyn A is lower in the primate cortex than the striatum, the greater  $\kappa$  affinity of dyn A (1–17) may imply that the increase in  $\kappa$  binding in primate cortex is related to this shift in the dyn A domain.

Prodynorphin is expressed and processed differently in the primate brain compared to the rodent, and is expressed differently within discrete systems of the primate brain. Brain prodynorphin processing is thus apparently not uniform across neural systems, nor across different species. The complexity of prodynorphin expression and processing, and its unique opiate receptor profile, needs to be the subject of future research to clarify its role in motor and higher cortical functions.

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### References

1. Chavkin C., James I. F. and Goldstein, A. Dynorphin is a specific endogenous ligand of the  $\kappa$  opioid receptor. *Science* 1982; 215: 413–415.

2. Docherty, K. and Steiner, D. F. Post-translational proteolysis in polypeptide hormone biosynthesis. *Ann. Rev. Physiol.* 1982; 44: 625-638.
3. Dores, R. M. and Akil, H. Steady state levels of pro-dynorphin-related end products in the striatum and substantia nigra of adult rhesus monkey. *Peptides*; 6 Suppl 1985; 2: 143-148.
4. Fallon, J. H. and Leslie, F. M. Distribution of dynorphin and enkephalin peptides in the rat brain. *J. Comp. Neurol.* 1986; 249: 293-336.
5. Kakidani, H., Furutani, Y., Takahashi, H. et al. Cloning and sequence analysis of cDNA for porcine  $\beta$ -neo-endorphin/dynorphin precursor. *Nature (London)* 1982; 298: 245-249.
6. Kangawa, K., Minamino, N., Chino, N., Sakakibara, S. and Matsuo, H. The complete amino acid sequence of  $\alpha$ -neo-endorphin. *Biochem. Biophys. Res. Commun.* 1981; 99: 871-878.
7. Khachaturian, H., Lewis, M. E., Haber, S. N., Houghten, R. A., Akil, H. and Watson, S. J. Prodynorphin peptide immunocytochemistry in rhesus monkey brain. *Peptides*; 6 Suppl 1985; 2: 155-166.
8. Khachaturian, H., Watson, S. J., Lewis, M. E., Coy, D., Goldstein, A. and Akil, H. Dynorphin immunocytochemistry in the rat central nervous system. *Peptides* 1982; 3: 941-954.
9. Leslie, F. M. Methods used for the study of opioid receptors. *Pharmacol. Review* 1987; 39: 197-249.
10. Lewis, M. E., Khachaturian, H., Akil, H. and Watson, S. J. Anatomical relationship between opioid peptides and receptors in rhesus monkey brain. *Brain Res. Bull. Dec.* 1984; 13 (6): 801-812.
11. Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H. and Watson, S. J. Anatomy of CNS opioid receptors. *Trends Neurosci.* 1988; 11: 308-314.
12. McLean, S., Rothman, R. B., Jacobson, A. E., Rice, K. C. and Herkenham, M. Distribution of opiate receptor subtypes and enkephalin and dynorphin immunoreactivity in the hippocampus of squirrel, guinea pig, rat and hamster. *J. Comp. Neurol.* 1987; 255: 497-510.
13. Minamino, N., Kangawa, K., Chino, N., Sakakibara, S. and Matsuo, H.  $\beta$ -neo-endorphin, a new hypothalamic "big" leu-enkephalin of porcine origin: its purification and the complete amino acid sequence. *Biochem. Biophys. Res. Commun.* 1981; 99: 864-870.
14. Minamino, N., Kangawa, K., Fukuda, A. and Matsuo, H. A new opioid octapeptide related to dynorphin from porcine hypothalamus. *Biochem. Biophys. Res. Commun.* 1980; 95: 1475-1481.
15. Nakao, K., Suda, M., Sakamoto, M. et al. Leuorphan is a novel endogenous opioid peptide derived from pro-enkephalin B. *Biochem. Biophys. Res. Commun.* 1983; 117: 695-701.
16. Neil, A., Terenius, L., Ternes, J. W., Ehrman, R. N. and O'Brien, C. P. Opiate receptors, neuropeptides in CNS and CSF of two *Macaca* species with different responsiveness to opiates. *Eur. J. Pharmacol. Mar.* 11; 1986; 122(1): 143-147.
17. Ramsdell, C. and Meador-Woodruff, J. H. Expression of prodynorphin-derived peptides and mRNA in guinea pig cortex. *Neuropeptides* 1993; 25: 131-138.
18. Seizinger, B. R., Holt, V. and Herz, A. Evidence for the occurrence of the opioid octapeptide dynorphin (1-8) in the neurointermediate pituitary of rats. *Biochem. Biophys. Res. Commun.* 1981; 102: 197-205.
19. Slater, P. and Cross, A. J. Autoradiographic distribution of dynorphin<sub>1-9</sub> binding sites in primate brain. *Neuropeptides* 1986; 8: 71-76.
20. Trujillo, K. A., Day, R. and Akil, H. Regulation of striatonigral prodynorphin peptides by dopaminergic agents. *Brain Res.* 1990; 518: 244-256.
21. Von Bonin, G. and Bailey, P. *The Neocortex Of Macaca Mulatta*. The University of Illinois Press, Urbana, IL, 1947.
22. Watson, S. J. and Akil, H. Immunocytochemistry of peptides. In: Barker, J. L., and McKelvey, J. F. (eds) *Current Methods in Cellular Neurobiology*. Wiley, New York, 1982: 111-131.