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## Appendix I

The percent [D-Arg<sup>2</sup>]-Kyotorphin remaining in the nasal perfusate  
containing various enhancers.

The percent remaining of [ D-Arg<sup>2</sup> ]-Kyotorphin in the nasal perfusate . Comparison of the decreasing profiles between the first hour of perfusion (with enhancer) and the second hour of perfusion (with enhancer).

Data: mean±SD (n=3-8 rats/group)

Isotonic saline pH 6.0

Subject No.	1 <sup>st</sup> hour.(min)					k	r <sup>2</sup>	2 <sup>nd</sup> hour.(min.)					k	r <sup>2</sup>	%ΔC
	0	15	30	45	60			0	15	30	45	60			
1	100.0	99.9	101.3	105.1	101.6	-	-	100.0	95.8	98.2	96.0	90.5	-	-	-10.93
2	100.0	102.6	102.0	102.8	109.8	-	-	100.0	100.7	100.9	102.7	99.5	-	-	-9.38
3	100.0	94.9	93.0	98.7	95.2	-	-	100.0	103.0	102.5	105.7	103.1	-	-	+8.3
4	100.0	96.1	101.7	102.6	95.7	-	-	100.0	98.7	93.4	95.4	97.4	-	-	+1.78
5	100.0	91.8	94.3	90.2	90.9	-	-	100.0	95.9	95.2	102.6	95.6	-	-	+5.17
6	100.0	92.7	94.9	88.5	91.0	-	-	100.0	97.0	95.3	90.8	97.7	-	-	+7.36
7	100.0	100.5	94.3	96.0	95.7	-	-	100.0	97.2	95.8	96.2	93.6	-	-	-2.19
8	100.0	98.7	101.4	97.5	104.8	-	-	100.0	91.6	94.9	93.3	90.5	-	-	-13.65
mean	100.0	97.1	97.7	97.7	98.1	-	-	100.0	97.5	97.0	97.8	96.0	-	-	-1.69
± SD	0.0	3.9	3.9	6.0	6.7	-	-	0.0	3.4	3.2	5.2	4.4	-	-	8.69

0.5% CSG pH 6.0

Subject No.	1 <sup>st</sup> hour.(min)					k	r <sup>2</sup>	2 <sup>nd</sup> hour.(min.)					k	r <sup>2</sup>	%ΔC
	0	15	30	45	60			0	15	30	45	60			
1	100.0	96.4	89.1	84.1	83.8	0.00326	0.9402	100.0	92.9	90.2	92.9	88.6	-	-	+5.73
2	100.0	98.5	96.9	89.1	84.9	0.00318	0.8389	100.0	94.8	94.9	95.6	92.3	-	-	+8.72
3	100.0	96.4	94.9	88.0	82.6	0.00343	0.9240	100.0	96.9	97.8	98.2	94.1	-	-	+13.92
4	100.0	98.8	96.5	87.0	81.3	0.00368	0.8922	100.0	100.2	93.5	95.1	90.7	-	-	+11.56
mean	100.0	97.5	94.3	87.0	83.2	0.00339	0.8988	100.0	96.2	94.1	95.5	91.4	-	-	+9.98
± SD	0.0	1.3	3.6	2.2	1.6	0.00022	0.0446	0.0	3.2	3.1	2.2	2.4	-	-	5.54

0.1% CSG pH 6.0

Subject No.	1 <sup>st</sup> hour.(min)					k	r <sup>2</sup>	2 <sup>nd</sup> hour.(min.)					k	r <sup>2</sup>	%ΔC
	0	15	30	45	60			0	15	30	45	60			
1	100.0	90.8	85.8	85.6	83.8	0.00326	0.7932	100.0	91.6	92.4	92.4	99.0	-	-	+18.14
2	100.0	92.6	87.1	86.1	80.6	0.00435	0.9846	100.0	101.9	98.0	91.0	89.6	-	-	+12.89
3	100.0	101.4	97.2	96.5	86.6	0.00124	0.3526	100.0	99.6	94.7	99.1	99.9	-	-	+3.42
mean	100.0	95.0	90.0	89.4	83.7	0.00295	0.71013	100.0	97.7	95.0	94.2	96.2	-	-	+11.48
± SD	0.0	5.7	6.3	6.1	3.0	0.00158	0.32408	0.0	5.4	2.8	4.3	5.7	-	-	7.46

0.5% CSJ pH 6.0

Subject No.	1 <sup>st</sup> hour.(min)					k	r <sup>2</sup>	2 <sup>nd</sup> hour.(min.)					k	r <sup>2</sup>	%ΔC
	0	15	30	45	60			0	15	30	45	60			
1	100.0	104.9	99.4	96.2	86.0	0.00260	0.6797	100.0	97.6	97.5	96.1	96.1	-	-	+11.74
2	100.0	101.3	88.9	88.1	78.2	0.00422	0.8874	100.0	96.9	93.6	90.6	93.6	-	-	+19.69
3	100.0	91.4	82.5	56.3	60.6	0.00981	0.8727	100.0	99.0	92.5	96.2	87.4	-	-	+44.22
4	100.0	97.7	87.8	85.6	88.0	0.00258	0.7581	100.0	103.6	101.4	97.4	103.0	-	-	+17.05
mean	100.0	98.8	89.7	81.5	78.2	0.00480	0.79948	100.0	99.3	96.3	95.1	95.0	-	-	+23.18
± SD	0.0	5.7	7.1	17.4	12.5	0.0034	0.0986	0.0	3.0	4.1	3.0	6.4	-	-	14.42

0.1% CSJ pH 6.0

Subject No.	1 <sup>st</sup> hour.(min)					k	r <sup>2</sup>	2 <sup>nd</sup> hour.(min.)					k	r <sup>2</sup>	%ΔC
	0	15	30	45	60			0	15	30	45	60			
1	100.0	98.8	87.6	78.8	75.6	0.00524	0.9504	100.0	97.9	96.9	95.8	96.5	-	-	+27.65
2	100.0	97.1	89.3	88.8	82.2	0.00320	0.9514	100.0	100.8	99.3	97.5	93.1	-	-	+13.26
3	100.0	101.1	88.1	83.9	80.6	0.00413	0.9067	100.0	106.8	102.0	102.0	103.0	-	-	+17.79
4	100.0	91.5	83.2	75.4	71.6	0.00573	0.9584	100.0	97.9	98.6	100.8	97.0	-	-	+35.47
mean	100.0	97.1	87.0	81.7	77.5	0.00458	0.9417	100.0	100.9	99.2	99.0	97.4	-	-	+26.04
± SD	0.0	4.1	2.7	5.8	4.8	0.00114	0.0236	0.0	4.2	2.1	2.9	4.1	-	-	9.27

0.1% LCC pH 6.0

Subject No.	1 <sup>st</sup> hour.(min)					k	r <sup>2</sup>	2 <sup>nd</sup> hour.(min.)					k	r <sup>2</sup>	%ΔC
	0	15	30	45	60			0	15	30	45	60			
1	100.0	84.2	78.4	72.4	64.8	0.00680	0.9744	100.0	90.3	85.8	80.7	74.8	-	-	+15.43
2	100.0	87.0	76.1	77.1	66.2	0.00631	0.9287	100.0	98.4	95.2	90.3	85.5	-	-	+29.15
3	100.0	99.9	89.7	87.6	80.0	0.00386	0.9312	100.0	92.9	102.9	95.9	91.2	-	-	+14.00
mean	100.0	90.4	81.4	79.0	70.3	0.00566	0.9448	100.0	93.9	94.6	89.0	83.8	-	-	+19.53
± SD	0.0	8.4	7.3	7.8	8.4	0.00158	0.0257	0.0	4.1	8.5	7.7	8.3	-	-	8.37

1.25% DM-β-CD pH 6.0

Subject No.	1 <sup>st</sup> hour.(min)					k	r <sup>2</sup>	2 <sup>nd</sup> hour.(min.)					k	r <sup>2</sup>	%ΔC
	0	15	30	45	60			0	15	30	45	60			
1	100.0	92.6	85.5	82.7	80.5	0.00366	0.9495	100.0	98.2	102.0	87.5	87.7	-	-	+8.94
2	100.0	90.3	88.4	82.2	75.9	0.00430	0.9707	100.0	96.2	93.4	92.6	90.9	-	-	+19.76
3	100.0	92.3	91.3	83.5	73.8	0.00472	0.9345	100.0	93.5	91.2	89.2	88.2	-	-	+19.51
4	100.0	103.4	96.4	95.8	91.0	0.00177	0.7558	100.0	104.2	96.4	102.0	101.7	-	-	+11.76
mean	100.0	94.7	90.4	86.0	80.3	0.00361	0.9026	100.0	98.0	95.7	92.8	92.1	-	-	+14.99
± SD	0.0	6.0	4.6	6.5	7.7	0.00130	0.0990	0.0	4.5	4.7	6.5	6.5	-	-	5.48

5.0 % HP-β-CD pH 6.0

Subject No.	1 <sup>st</sup> hour.(min)					k	r <sup>2</sup>	2 <sup>nd</sup> hour.(min.)					k	r <sup>2</sup>	%ΔC
	0	15	30	45	60			0	15	30	45	60			
1	100.0	99.6	103.9	101.2	97.4	-	-	100.0	98.2	100.2	105.0	105.9	-	-	+8.73
2	100.0	97.4	98.8	98.7	100.5	-	-	100.0	100.6	96.9	110.0	107.8	-	-	+7.26
3	100.0	95.5	95.0	96.5	97.0	-	-	100.0	99.7	94.3	100.3	100.9	-	-	+4.02
4	100.0	92.3	85.0	100.9	100.3	-	-	100.0	99.6	96.1	100.1	102.9	-	-	+2.59
5	100.0	103.0	108.4	107.5	102.5	-	-	100.0	95.8	94.8	92.5	92.1	-	-	-10.15
6	100.0	96.7	97.4	97.6	97.8	-	-	100.0	103.8	101.1	103.8	103.3	-	-	+5.62
7	100.0	100.7	99.5	100.6	102.1	-	-	100.0	94.8	92.1	94.9	93.9	-	-	-8.03
mean	100.0	97.9	98.3	100.4	99.6	-	-	100.0	98.9	96.5	100.9	101.0	-	-	+1.44
± SD	0.0	3.5	7.3	3.6	2.3	-	-	0.0	3.0	3.2	6.0	5.9	-	-	7.49



## Appendix II

Statistical analysis of the percent [D-Arg<sup>2</sup>]-Kyotorphin remaining in the nasal perfusate and the apparent rate constant after the first period of nasal perfusion in the presence of various enhancers.

## Appendix II a

% [D-Arg<sup>2</sup>]-Kyotorphin remaining in the nasal perfusate at the end of the first perfusion period in the presence of various enhancers.

One-way ANOVA

## Means

Mean # 1 =	98.08749	Control
Mean # 2 =	83.15001	0.5% CS G
Mean # 3 =	78.2	0.5% CS J
Mean # 4 =	70.3334	0.1% LCC
Mean # 5 =	80.3	1.25% DM-β-CD
Mean # 6 =	99.67143	5.0% HP-β-CD

Source	df	SS	MS	F	Prob.
Between	5	3406.016	681.2031	14.35883	.0001
Within	24	1138.594	47.44141		
Total	29	4544.61			

Duncan's multiple rang test

MS Error = 47.44141

df Error = 24

Significance level = 0.05

Least Significant Ranges	
LSR for Mean 6 vs Mean 1	11.0237
LSR for Mean 6 vs Mean 2	9.8296
LSR for Mean 6 vs Mean 3	9.6159
LSR for Mean 6 vs Mean 4	9.3717
LSR for Mean 6 vs Mean 5	7.3603
LSR for Mean 5 vs Mean 1	10.6172
LSR for Mean 5 vs Mean 2	9.3949
LSR for Mean 5 vs Mean 3	9.1563
LSR for Mean 5 vs Mean 4	8.7089
LSR for Mean 5 vs Mean 1	11.7174
LSR for Mean 4 vs Mean 2	10.5727
LSR for Mean 4 vs Mean 3	10.0561
LSR for Mean 3 vs Mean 2	11.4199
LSR for Mean 3 vs Mean 1	10.0561
LSR for Mean 2 vs Mean 1	10.8619

#### Means

Mean # 1	70.33334	0.1% LCC
Mean # 2	78.20	0.5% CS J
Mean # 3	80.3	1.25% DM- $\beta$ -CD
Mean # 4	83.15001	0.5% CS G
Mean # 5	98.08749	Control
Mean # 6	99.67143	5.0% HP- $\beta$ -CD

1      2      3      4      5      6

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## Appendix II b

% [D-Arg<sup>2</sup>]-Kyotorphin remaining in the nasal perfusate at the end of the first perfusion period in the presence of various enhancers.

One-way ANOVA

Means

Mean # 1 =	83.15001	0.5% CS G
Mean # 2 =	78.2	0.5% CS J
Mean # 3 =	70.33334	0.1% LCC
Mean # 4 =	80.3	1.25% DM- $\beta$ -CD

  

Source	df	SS	MS	F	Prob.
Between	3	299.875	99.95834	1.389818	.2973
Within	11	791.1406	71.92188		
Total	14	1091.016			

## Appendix IIc

The  $k_{obs}$  values which calculated from slope of the first perfusion period in the presence of various enhancers .

One-way ANOVA

Means

Mean # 1 =		0.0033875			0.5% CS G
Mean # 2 =		0.0048025			0.5% CS J
Mean # 3 =		0.0056567			0.1% LCC
Mean # 4 =		0.0036125			1.25% DM- $\beta$ -CD
Source	df	SS	MS	F	Prob.
Between	3	$1.1746 \times 10^{-5}$	$3.91537 \times 10^{-6}$	0.9484512	.5475
Within	11	$4.54098 \times 10^{-5}$	$4.12817 \times 10^{-6}$		
Total	14	$5.7156 \times 10^{-5}$			

### Appendix III

Statistical comparison between percent of [D-Arg<sup>2</sup>]-Kyotorphin remaining in the nasal perfusate at the end of the first and second perfusion periods.

Comparison between % [D-Arg<sup>2</sup>]-Kyotorphin remaining in nasal perfusates at the end of the first and second perfusion period for the control, 5.0% HP- $\beta$ -CD, 0.5% CS J, 0.5% CS G, 1.25% DM- $\beta$ -CD and 0.1% LCC-treated groups.

Paired t- test

### Control group

1 st hour

mean = 98.08794

standard deviation = 6.721169

standard error = 2.376292

2 nd hour

mean = 95.98749

standard deviation = 4.374107

standard error = 1.54648

Standard error of the difference = 3.091463

t = 0.6792895

p = 0.259389

Degrees of freedom = 7

### 5.0% HP- $\beta$ -CD

1 st hour

mean = 99.65714

standard deviation = 2.264996

standard error = 0.8560882

2 nd hour

mean = 100.9714

standard deviation = 5.898667

standard error = 2.229487

Standard error of the difference = 2.849681

t = -0.4612071

p = 0.330448

Degrees of freedom = 6

**0.5% CS J**

1 st hour

mean = 80.98

standard deviation = 12.46201

standard error = 5.573181

2 nd hour

mean = 93.72

standard deviation = 6.304451

standard error = 2.819436

Standard error of the difference = 4.918496

t = -2.590224

p = 0.030335

Degrees of freedom = 3

**0.5% CS G**

1 st hour

mean = 83.15001

standard deviation = 1.54995

standard error = 0.7749748

2 nd hour

mean = 91.425

standard deviation = 2.339928

standard error = 1.169964

Standard error of the difference = 1.429088

t = -5.790402

p = 0.005123

Degrees of freedom = 3



**1.25% DM- $\beta$ -CD**

1 st hour

mean = 80.3

standard deviation = 7.662434

standard error = 3.831217

2 nd hour

mean = 92.125

standard deviation = 6.536258

standard error = 3.268129

Standard error of the difference = 1.811249

t = -6.528641

p = 0.003651

Degrees of freedom = 3

**0.1% LCC**

1 st hour

mean = 70.33334

standard deviation = 8.400817

standard error = 4.850214

2 nd hour

mean = 83.83334

standard deviation = 8.326058

standard error = 4.807052

Standard error of the difference = 2.920618

t = -4.622309

p = 0.021877

Degrees of freedom = 2

## Appendix IV

Statistical analysis of percent change in % [D-Arg<sup>2</sup>]-Kyotorphin remaining in the nasal perfusate at the end of the second period relative to that of the first perfusion .

Percent change in % [D-Arg<sup>2</sup>]-Kyotorphin remaining in the nasal perfusate at the end of the second period relative to that of the first perfusion (% $\Delta$ C).

Comparison among the four reversible enhancers.

One-way ANOVA

Means

Mean # 1 =	9.9825	0.5% CS G
Mean # 2 =	23.175	0.5% CS J
Mean # 3 =	19.52667	0.1% LCC
Mean # 4 =	14.9925	1.25% DM- $\beta$ -CD

  

Source	df	SS	MS	F	Prob.
Between	3	383.8032	127.9344	1.579197	0.2497
Within	11	891.1358	81.01234		
Total	14	1274.939			

## Appendix V

The release profiles of LDH in the nasal perfusate in the presence and  
after removal of different enhancers

Concentration ( U/mL ) of lactate dehydrogenase ( LDH ) in the nasal perfusates. Comparison of the release profiles between the first hour of perfusion ( with enhancer ) and the second hour of perfusion ( without enhancer ).

Data : mean  $\pm$  SD ( n = 3-4 rats/group )

Control group	n	1 <sup>st</sup> hour ( Saline only )				2 <sup>nd</sup> hour ( Saline only )				% $\Delta$ C <sub>60</sub> [(C <sub>2</sub> -C <sub>1</sub> )/C <sub>1</sub> ]*100%
		(min) 15	30	45	60	(min) 15	30	45	60	
Isotonic saline pH 6	1	20	80.72	50.27	51.95		97.89	101.25	117.95	127.05
	2	53.33	35.25	36.45	37.65	13.33	20.48	21.2	61.92	64.46
	3	20	14.05	27.87	35.49	46.67	55.01	70.27	72.67	104.76
	4	40	20.65	34.33	28.24	40	13.98	40.88	28.24	0
	mean	33.33	37.67	37.23	38.33	33.33	46.84	58.40	70.20	74.07
$\pm$ SD	16.33	30.04	9.43	9.93	17.64	38.50	34.97	37.04	55.76	

	n	1 <sup>st</sup> hour ( with HP- $\beta$ -CD )				2 <sup>nd</sup> hour ( Saline only )				% $\Delta$ C <sub>60</sub> [(C <sub>2</sub> -C <sub>1</sub> )/C <sub>1</sub> ]*100%
		(min) 15	30	45	60	(min) 15	30	45	60	
5% w/v HP $\beta$ CD pH6.0	1	40	48.11	49.79	58.13	13.33	13.81	54.29	32.88	-43.44
	2	33.33	41.2	55.97	54.56	6.67	13.57	14.05	44.53	-18.38
	3	33.33	34.53	35.73	50.27	20	34.05	28.59	42.88	-14.70
	4	46.67	21.68	55.73	37.65	26.67	27.63	95.25	78.61	108.79
	mean	38.33	36.38	49.31	50.15	16.67	22.27	48.05	49.73	8.07
$\pm$ SD	6.39	11.26	9.49	8.93	8.61	10.24	35.60	19.93	68.35	

	n	1 <sup>st</sup> hour ( with DM-β-CD )				2 <sup>nd</sup> hour ( Saline only )				%ΔC <sub>60</sub> [(C <sub>2</sub> -C <sub>1</sub> )/C <sub>1</sub> ]*100%
		(min) 15	30	45	60	(min) 15	30	45	60	
1.25% w/v DMβCD pH6.0	1	440	533.82	629.31	653.22	20	53.66	121.21	69.92	-89.30
	2	446.67	367.26	380	386.39	33.33	47.21	47.99	55.46	-85.65
	3	333.33	312.09	503.91	505.71	40	47.32	61.43	49.13	-90.28
	4	393.33	526.4	475.1	496.41	60	80.98	115.65	130.92	-73.63
	mean	403.33	434.89	497.08	510.43	38.33	57.29	86.57	76.36	-84.72
± SD	52.35	112.27	102.83	109.53	16.67	16.08	37.26	37.40	7.65	

	n	1 <sup>st</sup> hour ( with LCC )				2 <sup>nd</sup> hour ( Saline only )				%ΔC <sub>60</sub> [(c <sub>2</sub> -c <sub>1</sub> )/c <sub>1</sub> ]*100%
		(min) 15	30	45	60	(min) 15	30	45	60	
0.1% w/v LCC pH6.0	1	706.67	795.15	911.25	1035.31	53.33	73.97	68.19	115.65	-88.83
	2	846.67	930.48	1089.55	1073.17	40	41.44	56.21	78.13	-92.72
	3	706.67	845.44	1014.96	1016.19	40	68.11	70.51	89.57	-91.19
	4	906.67	1325.97	1479.2	1492.93	60	88.83	105.28	125.55	-91.59
	mean	791.67	974.26	1,123.74	1,154.40	48.33	68.09	75.05	102.23	-91.08
± SD	101.16	241.03	248.00	226.93	10.00	19.79	21.11	22.10	1.64	

	n	1 <sup>st</sup> hour ( with CSJ )				2 <sup>nd</sup> hour ( Saline only )				%ΔC <sub>60</sub> [(C <sub>2</sub> -C <sub>1</sub> )/C <sub>1</sub> ]*100%
		(min) 15	30	45	60	(min) 15	30	45	60	
0.5% w/v CSJ pH6.0	1	66.67	194.42	310.96	422.86	86.67	121.41	143.42	179.16	-57.63
	2	86.67	248.08	325.51	470.98	46.67	127.43	129.53	131.71	-72.03
	3	153.33	335.83	494.71	549.69	100	128.29	150.41	186.27	-66.11
	4	46.67	174.09	256.97	267.96	66.67	81.08	95.76	117.37	-56.20
	mean	88.34	238.11	347.04	427.87	75.00	114.55	129.78	153.63	-62.99
	± SD	46.31	72.24	102.77	118.74	23.33	22.52	24.28	34.22	7.45

	n	1 <sup>st</sup> hour ( with CSG )				2 <sup>nd</sup> hour ( Saline only )				%ΔC <sub>60</sub> [(C <sub>2</sub> -C <sub>1</sub> )/C <sub>1</sub> ]*100%
		(min) 15	30	45	60	(min) 15	30	45	60	
0.1% w/v CSJ pH6.0	1	40	173.98	123.53	345.6	140	228.94	279.39	279.39	-19.16
	2	13.33	140.22	229.2	339.72	53.33	74.2	68.76	83.25	-75.49
	3	33.33	387.21	520.28	642.35	86.67	128.08	170.19	193.05	-69.95
	mean	28.89	233.80	291.00	442.56	93.33	143.74	172.78	185.23	-54.87
	± SD	13.88	133.92	205.47	173.05	43.72	78.55	105.34	98.30	31.05

	n	1 <sup>st</sup> hour (with CSG)				2 <sup>nd</sup> hour (Saline only)				%ΔC <sub>60</sub> [(C <sub>2</sub> -C <sub>1</sub> )/C <sub>1</sub> ]*100%
		(min) 15	30	45	60	(min) 15	30	45	60	
0.5% w/v	1	273.33	424.44	584.79	674.62	126.67	195.39	191.96	255.18	-62.17
CSG	2	80	201.3	351.29	390.53	33.33	40.54	54.55	68.8	-82.38
pH6.0	3	46.67	194.09	350.63	456.53	73.33	101.19	129.53	158.38	-65.31
	4	80	107.97	249.75	393.95	80	147.97	157.08	199.72	-49.30
	mean	120.00	231.95	384.12	478.91	78.33	121.27	133.28	170.52	-64.79
	± SD	103.42	135.15	142.04	133.96	38.25	66.15	58.37	78.56	13.62

	n	1 <sup>st</sup> hour (with CSG)				2 <sup>nd</sup> hour (Saline only)				%ΔC <sub>60</sub> [(C <sub>2</sub> -C <sub>1</sub> )/C <sub>1</sub> ]*100%
		(min) 15	30	45	60	(min) 15	30	45	60	
0.1% w/v	1	53.33	200.87	450.85	585.1	60	94.31	122.53	144.59	-75.29
CSG	2	60	214.31	357.85	463.87	193.33	296.48	181.38	204.43	-55.93
pH6.0	3	33.33	180.54	310.19	388.74	160	189.27	219.06	229.41	-40.99
	mean	48.89	198.57	372.96	479.24	137.78	193.35	174.32	192.81	-57.4
	± SD	13.88	17.00	71.54	99.08	69.39	101.15	48.65	43.59	17.20

%ΔC<sub>60</sub> = Percentage change in conc. LDH at the end of 2<sup>nd</sup> hour relative to that of 1<sup>st</sup> hour

$$= \Delta C_{60} / C_1 * 100\% = [(C_2 - C_1) / C_1] * 100\%$$



## Appendix VI

Statistical analysis of the LDH Content released in the nasal perfusate  
during the first perfusion period .

## Appendix VI a

The LDH content at 15 min of the first perfusion period of control and five enhancer-treated groups.

## One-way ANOVA

## Means

---

Mean # 1 =	33.3325	Control
Mean # 2 =	38.3325	5.0% HP- $\beta$ -CD
Mean # 3 =	403.3325	1.25% DM- $\beta$ -CD
Mean # 4 =	791.67	0.1% LCC
Mean # 5 =	88.33499	0.5% CS J
Mean # 6 =	120	0.5% CS G

---

Source	df	SS	MS	F	Prob.
Between	5	1806386	361277.3	82.98309	.0001
Within	18	78365.25	4353.625		
Total	23	1884752			

---

Duncan's multiple range test

MS Error = 4353.625

df Error = 18

Significance level = 0.05

Least Significant Ranges	
LSR where $p = 2$	68.2914
LSR where $p = 3$	102.9319
LSR where $p = 4$	105.9011
LSR where $p = 5$	107.8806
LSR where $p = 6$	109.5301

Means

---

Mean # 1 =	33.3325	Control
Mean # 2 =	38.3325	5.0% HP- $\beta$ -CD
Mean # 3 =	88.33499	0.5% CS J
Mean # 4 =	120	0.5% CS G
Mean # 5 =	403.3325	1.25% DM- $\beta$ -CD
Mean # 6 =	791.67	0.1% LCC

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1      2      3      4      5      6

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## Appendix VI b

The LDH content at the end of the first perfusion period in the presence of various enhancers

One-way ANOVA

Means

Mean # 1 =	38.33251	Control
Mean # 2 =	50.15251	5.0% HP- $\beta$ -CD
Mean # 3 =	510.4325	1.25% DM- $\beta$ -CD
Mean # 4 =	1154.4	0.1% LCC
Mean # 5 =	427.8725	0.5% CS J
Mean # 6 =	478.9076	0.5% CS G

  

Source	df	SS	MS	F	Prob.
Between	5	3320959	664191.7	41.63602	.0001
Within	18	287142	15952.33		
Total	23	3608101			

Duncan's multiple range test

MS Error = 15952.33      df Error = 18

Significance level = 0.05

Least Significant Ranges	
LSR where p = 2	130.7231
LSR where p = 3	197.0320
LSR where p = 4	202.7156
LSR where p = 5	206.5047
LSR where p = 6	209.6622

Means

---

Mean # 1 =	38.33251	Control
Mean # 2 =	50.15251	5.0% HP- $\beta$ -CD
Mean # 3 =	427.8725	0.5% CS J
Mean # 4 =	478.9076	0.5% CS G
Mean # 5 =	510.4325	1.25% DM- $\beta$ -CD
Mean # 6 =	1154.4	0.1% LCC

---

1      2      3      4      5      6

## Appendix VI c

The LDH content at the end of the second perfusion period in which the enhancer was removal from the nasal cavity.

One-way ANOVA

Means

Mean # 1 =	70.195	Control
Mean # 2 =	49.725	5.0% HP- $\beta$ -CD
Mean # 3 =	76.3575	1.25% DM- $\beta$ -CD
Mean # 4 =	102.225	0.1% LCC
Mean # 5 =	153.6275	0.5% CS J
Mean # 6 =	170.52	0.5% CS G

  

Source	df	SS	MS	F	Prob.
Between	5	46973.21	9394.641	5.125039	0.0046
Within	18	32995.56	1833.087		
Total	23	79968.77			

Duncan's multiple range test

MS Error = 1833.087

df Error = 18

Significance level = 0.05

Least Significant Ranges	
LSR for Mean 6 vs Mean 1	71.0722
LSR for Mean 6 vs Mean 2	70.0018
LSR for Mean 6 vs Mean 3	68.7174
LSR for Mean 6 vs Mean 4	66.7907
LSR for Mean 6 vs Mean 5	44.3131
LSR for Mean 5 vs Mean 1	70.0018
LSR for Mean 5 vs Mean 2	68.7174
LSR for Mean 5 vs Mean 3	66.7907
LSR for Mean 5 vs Mean 4	44.3131
LSR for Mean 5 vs Mean 1	68.7174
LSR for Mean 4 vs Mean 2	66.7907
LSR for Mean 4 vs Mean 3	44.3131
LSR for Mean 3 vs Mean 2	66.7907
LSR for Mean 3 vs Mean 1	44.3131
LSR for Mean 2 vs Mean 1	44.3131

#### Means

Mean # 1	49.725	5.0% HP- $\beta$ -CD
Mean # 2	70.195	Control
Mean # 3	76.3575	1.25% DM- $\beta$ -CD
Mean # 4	102.225	0.1% LCC
Mean # 5	153.6275	0.5% CS J
Mean # 6	170.52	0.5% CS G

1      2      3      4      5      6

## Appendix VII

Statistical comparison between the LDH content in the nasal perfusate at the end of the first and second perfusion periods.



Comparison between the LDH content at the end of the first and second perfusion periods for the control, 5.0% HP- $\beta$ -CD, 0.5% CS J, 0.5% CS G, 1.25% DM- $\beta$ -CD and 0.1% LCC-treated groups.

Paired t- test

**Control group**

1 st hour

mean = 38.33251

standard deviation = 9.930414

standard error = 4.965207

2 nd hour

mean = 70.195

standard deviation = 37.03764

standard error = 18.51882

Standard error of the difference = 13.74321

t = -2.318418

p = 0.051617

Degrees of freedom = 3

**5.0% HP- $\beta$ -CD**

1 st hour

mean = 50.15251

standard deviation = 8.932928

standard error = 4.466464

2 nd hour

mean = 49.725

standard deviation = 19.93271

standard error = 9.966353

Standard error of the difference = 14.34621

t = 0.029799

p = 0.489050

Degrees of freedom = 3

**0.5% CS J**

1 st hour

mean = 427.8725

standard deviation = 118.7363

standard error = 59.36816

2 nd hour

mean = 153.6275

standard deviation = 34.21706

standard error = 17.10853

Standard error of the difference = 48.65183

t = 5.636891

p = 0.005523

Degrees of freedom = 3

**0.5% CS G**

1 st hour

mean = 478.9076

standard deviation = 133.9557

standard error = 66.97785

2 nd hour

mean = 170.52

standard deviation = 78.55846

standard error = 39.27923

Standard error of the difference = 46.23083

t = 6.670605

p = 0.003435

Degrees of freedom = 3

**1.25% DM- $\beta$ -CD**

1 st hour

mean = 510.4325

standard deviation = 109.5349

standard error = 54.76745

2 nd hour

mean = 76.3575

standard deviation = 37.40122

standard error = 18.70061

Standard error of the difference = 56.36003

t = 7.701824

p = 0.002275

Degrees of freedom = 3

**0.1% LCC**

1 st hour

mean = 1154.4

standard deviation = 226.9255

standard error = 113.4627

2 nd hour

mean = 102.225

standard deviation = 22.09824

standard error = 11.04912

Standard error of the difference = 106.4356

t = 9.885553

p = 0.001101

Degrees of freedom = 3

## Appendix VIII

Statistical analysis of the percent change in LDH content in the nasal perfusate at the end of the second perfusion period relative to that of the first period

The percent change in the LDH content in the nasal perfusate at the end of the second perfusion period relative to that of the first period (% $\Delta$ C)

Comparison between the four reversible enhancers.

One-way ANOVA

Means

Mean # 1 =	-84.71501	1.25% DM- $\beta$ -CD
Mean # 2 =	-91.08249	0.1% LCC
Mean # 3 =	-62.9925	0.5% CS J
Mean # 4 =	-64.79	0.5% CS G

  

Source	df	SS	MS	F	Prob.
Between	3	2392.985	797.6615	10.55886	0.0015
Within	12	906.5312	75.54428		
Total	15	3299.516			

Duncan's multiple range test

MS Error = 75.54428

df Error = 12

Significance level = 0.05

Least Significant Ranges	
LSR where $p = 2$	13.3851
LSR where $p = 3$	14.0370
LSR where $p = 4$	14.4716

Means

---

Mean # 1 =	-91.08249	0.1% LCC
Mean # 2 =	-84.71501	1.25% DM- $\beta$ -CD
Mean # 3 =	-64.79	0.5% CS G
Mean # 4 =	-62.9925	0.5% CS J

1    2    3    4

---

## Appendix IX

Comparison of the difference between 0.1% and 0.5% CS J

## Appendix IXa

% [D-Arg<sup>2</sup>]-Kyotorphin remaining at the end of the first perfusion period in the presence of 0.1 % and 0.5% CS J

Unpaired t test

0.1% CS J

mean	= 77.5
standard deviation	= 4.834501
standard error	= 2.417251

0.5% CS J

mean	= 78.2
standard deviation	= 12.47187
standard error	= 6.235935
pooled SE	= 6.688048
t	= -0.1046639
p (probability)	= 0.920054
degrees of freedom	= 6



## Appendix IXb

% [D-Arg<sup>2</sup>]-Kyotorphin remaining at the end of the second perfusion period in the presence of 0.1 % and 0.5% CS J

Unpaired t test

## 0.1% CS J

mean	= 97.4
standard deviation	= 4.115836
standard error	= 2.057918

## 0.5% CS J

mean	= 95.025
standard deviation	= 6.453056
standard error	= 3.226528
pooled SE	= 3.826945
t	= 0.6205995
p (probability)	= 0.557685
degrees of freedom	= 6

## Appendix IXc

% percent change in the % [D-Arg<sup>2</sup>]-Kyotorphin remaining at the end of the second perfusion period relative to that of the first period ( % $\Delta C_{\text{Ky}}$ ) in the presence of 0.1 % and 0.5% CS J

Unpaired t test

## 0.1% CS J

mean = 26.0425

standard deviation = 9.271969

standard error = 4.635985

## 0.5% CS J

mean = 23.175

standard deviation = 14.41426

standard error = 7.207128

pooled SE = 8.569424

t = 0.33462

p (probability) = 0.749300

degrees of freedom = 6

## Appendix IXd

LDH content in the nasal perfusate at the end of the first perfusion period in the presence of 0.1 % and 0.5% CS J

Unpaired t test

0.1% CS J

mean = 442.5567

standard deviation = 173.0511

standard error = 99.91111

0.5% CS J

mean = 427.8725

standard deviation = 118.7363

standard error = 59.36816

pooled SE = 109.1878

t = 0.1344852

p (probability) = 0.898265

degrees of freedom = 5

## Appendix IXe

LDH content in the nasal perfusate at the end of the second perfusion period in the presence of 0.1 % and 0.5% CS J

Unpaired t test

0.1% CS J

mean	= 185.23
standard deviation	= 98.30358
standard error	= 56.7556

0.5% CS J

mean	= 153.6275
standard deviation	= 34.21706
standard error	= 17.10853
pooled SE	= 51.61997
t	= 0.6122145
p (probability)	= 0.567164
degrees of freedom	= 5

## Appendix IXf

% percent change in the LDH content at the end of the second perfusion period relative to that of the first period ( $\% \Delta C_{LDH}$ ) in the presence of 0.1 % and 0.5% CS J

Unpaired t test

## 0.1% CS J

mean = -54.86667

standard deviation = 31.04671

standard error = 17.92482

## 0.5% CS J

mean = -62.9925

standard deviation = 7.445112

standard error = 3.722556

pooled SE = 15.63042

t = 0.5198733

p (probability) = 0.62534

degrees of freedom = 5

## Appendix X

Comparison of the difference between 0.1% and 0.5% CS G

## Appendix Xa

% [D-Arg<sup>2</sup>]-Kyotorphin remaining at the end of the first perfusion period in the presence of 0.1 % and 0.5% CS G

Unpaired t test

0.1% CS G

mean = 86.0  
standard deviation = 9.689163  
standard error = 5.594041

0.5% CS G

mean = 83.15001  
standard deviation = 1.54995  
standard error = 0.7749748  
pooled SE = 4.76929  
t = 0.5975713  
p (probability) = 0.576158  
degrees of freedom = 5

## Appendix Xb

% [D-Arg<sup>2</sup>]-Kyotorphin remaining at the end of the second perfusion period in the presence of 0.1 % and 0.5% CS G

Unpaired t test

0.1% CS G

mean	= 95.5
standard deviation	= 6.856367
standard error	= 3.958525

0.5% CS G

mean	= 91.425
standard deviation	= 2.339928
standard error	= 1.169964
pooled SE	= 3.589608
t	= 1.135221
p (probability)	= 0.307753
degrees of freedom	= 5



## Appendix Xc

% percent change in the % [D-Arg<sup>2</sup>]-Kyotorphin remaining at the end of the second perfusion period relative to that of the first period ( % $\Delta C_{Ky0}$ ) in the presence of 0.1 % and 0.5% CS G

Unpaired t test

## 0.1% CS G

mean	= 11.48333
standard deviation	= 7.460139
standard error	= 4.307113

## 0.5% CS G

mean	= 9.9825
standard deviation	= 3.543542
standard error	= 1.771771
pooled SE	= 4.169016
t	= 0.3599968
p (probability)	= 0.733556
degrees of freedom	= 5

## Appendix Xd

LDH content in the nasal perfusate at the end of the first perfusion period in the presence of 0.1 % and 0.5% CS G

Unpaired t test

0.1% CS G

mean = 479.2367

standard deviation = 99.07778

standard error = 57.20259

0.5% CS G

mean = 478.9076

standard deviation = 133.9557

standard error = 66.97785

pooled SE = 92.57936

t = 0.003555135

p (probability) = 0.997301

degrees of freedom = 5

## Appendix Xe

LDH content in the nasal perfusate at the end of the second perfusion period in the presence of 0.1 % and 0.5% CS G

Unpaired t test

## 0.1% CS G

mean	= 192.81
standard deviation	= 43.5876
standard error	= 25.16531

## 0.5% CS G

mean	= 170.52
standard deviation	= 78.55846
standard error	= 39.27923
pooled SE	= 51.02261
t	= 0.4368654
p (probability)	= 0.680432
degrees of freedom	= 5

## Appendix Xf

% percent change in the LDH content at the end of the second perfusion period relative to that of the first period ( $\% \Delta C_{LDH}$ ) in the presence of 0.1 % and 0.5% CS G

Unpaired t test

## 0.1% CS G

mean = -57.40334

standard deviation = 17.1974

standard error = 9.928922

## 0.5% CS G

mean = --64.79

standard deviation = 13.61965

standard error = 6.809824

pooled SE = 11.57288

t = 0.6382729

p (probability) = 0.551379

degrees of freedom = 5

## Appendix XI

Statistical analysis of the correlation between the % [D-Arg<sup>2</sup>]-Kyotorphin remaining in the nasal perfusate and the LDH content at the end of the first perfusion period.

Correlation data of the % [D-Arg<sup>2</sup>]-Kyotorphin remaining in the nasal perfusate and the LDH content at the end of the first perfusion period

	LDH content	% dipeptide remaining
Control	38.33	98.1
5% HP-β-CD	50.15	99.6
1.25%DM-β-CD	510.40	80.3
0.1% LCC	1154.40	70.33
0.5% CS J	427.87	78.2
0.5% CS G	478.91	83.2

$$Y = -0.0262x + 96.58$$

$$r^2 = 0.8492$$

Test of Zero correlation

$$H_0 : \rho = 0$$

$$H_a : \rho \neq 0$$

Where  $\rho$  is the true correlation coefficient, estimate by  $r$

$$t_{n-2} = \frac{|r| \sqrt{n-2}}{\sqrt{1-r^2}}$$

When  $r = -0.9215$

$$\begin{aligned} t_{n-2} &= \frac{|-0.9215| \sqrt{6-2}}{\sqrt{1-0.8492}} \\ &= 4.746 \end{aligned}$$

From the correlation coefficient at 5% level of significance,  $t_{0.025} (df = 4)$  is 2.776. Therefore, there is significant correlation between the LDH content and % dipeptide remaining at the end of the first perfusion period ( $p < 0.05$ )

## Appendix XII

Statistical analysis of the correlation between the percent change in the % [D-Arg<sup>2</sup>]-Kyotorphin remaining in the nasal perfusate and the percent change in the LDH content at the end of the second perfusion period relative to that of the first period (% $\Delta$ C dipeptide remaining and % $\Delta$ C LDH content).

Correlation data of the percent change in % [D-Arg<sup>2</sup>]-Kyotorphin remaining in the nasal perfusate and the LDH content at the end of the second perfusion period relative to that of the first period.(%Δc dipeptide remaining and %Δc LDH content )

	%Δc LDH content	%Δc dipeptide remaining
1.25%DM-β-CD	-84.72	14.99
0.1% LCC	-91.08	19.53
0.5% CS J	-62.99	23.18
0.5% CS G	-64.79	9.98

$$y = -0.031x + 14.525 \quad r^2 = 0.0061$$

Test of Zero correlation

$$H_0 : \rho = 0$$

$$H_a : \rho \neq 0$$

Where  $\rho$  is the true correlation coefficient, estimate by  $r$

$$t_{n-2} = \frac{|r| \sqrt{n-2}}{\sqrt{1-r^2}}$$

When  $r = -0.0781$

$$\begin{aligned} t_{n-2} &= \frac{|-0.0781| \sqrt{6-2}}{\sqrt{1-0.0061}} \\ &= 0.1567 \end{aligned}$$

From the correlation coefficient at 5% level of significance,  $t_{0.025} (df = 4)$  is 2.776. Therefore, there is no significant correlation between %  $\Delta C_{LDH}$  and %  $\Delta C_{Kyo}$  ( $p > 0.05$ )



## Appendix XIII

### Demographic Data

## Demographic Data

Subject No.	Age (yr)	Height (cm)	Weight (kg)
1	21	161	48
2	25	160	52
3	29	180	95
4	29	167	83
5	22	169	65
6	26	170	56
7☆	24	165	52
8	25	160	55



It reported that nasal sores, irritation were occurred in this subject treated with 300 IU sCT in 0.53% CS G in the second week.

## Appendix XIV

Percent plasma calcium level relative to the initial value after nasal  
administration of sCT with and without Enhancers

The % calcium plasma level relative to the initial point following nasal administration of 300 units sCT( isotonic pH 6 )

subject no.	Times													AUC <sub>(0-9hr)</sub>
	0	0.5	1	1.5	2	2.5	3	4	5	6	7	8	9	
1	100.0	99.1	93.8	92.6	89.7	90.0	96.2	97.4	94.7	95.6	98.2	95.9	98.2	860.65
2	100.0	102.2	102.8	100.0	99.1	103.4	98.8	101.9	97.2	98.2	97.8	100.3	93.2	894.85
3	100.0	100.6	100.6	100.3	100.3	94.9	100.9	99.0	96.2	98.1	98.7	96.2	101.8	888.12
4	100.0	99.4	99.7	97.8	99.4	97.5	98.2	109.0	100.6	96.3	96.3	96.9	99.4	894.35
5	100.0	97.9	100.7	101.9	97.0	97.9	99.1	99.7	96.7	91.8	97.3	91.5	94.3	871.18
6	100.0	100.9	97.1	99.0	102.2	100.3	100.9	103.2	103.2	97.4	100.6	100.9	105.4	908.43
7	100.0	99.1	99.4	101.2	99.1	99.1	99.4	98.2	98.5	96.4	96.4	100.0	98.5	887.25
8	100.0	92.8	93.1	100.6	90.7	89.2	95.2	94.9	89.2	93.4	94.0	93.7	99.1	844.35
mean	100.0	99.0	98.4	99.2	97.2	96.5	98.6	100.4	97.0	95.9	97.4	96.9	98.7	881.15
%CV	0.03	2.87	3.5	2.94	4.69	5.12	2.08	4.32	4.28	2.35	2.01	3.45	3.94	2.38
SEM	0.01	1.01	1.22	1.03	1.61	1.75	0.73	1.53	1.47	0.8	0.69	1.18	1.37	7.40

The % plasma calcium level relative to the initial point following nasal administration of 300 units sCT.

( with 1.25% DM- $\beta$ -CD)

subject no.	Times													AUC <sub>(0-9hr)</sub>
	0	0.5	1	1.5	2	2.5	3	4	5	6	7	8	9	
1	100.0	95.1	101.1	98.9	97.5	90.5	106.0	98.2	106.7	103.2	98.6	102.1	99.3	904.50
2	100.0	100.3	99.1	101.6	101.0	102.9	100.3	101.3	98.1	101.6	103.8	103.2	103.2	912.28
3	100.0	100.3	97.1	96.2	94.1	95.9	98.5	98.2	100.0	97.6	96.2	94.7	104.5	879.62
4	100.0	98.8	101.3	100.3	104.1	91.9	90.0	100.6	103.8	97.8	98.4	100.0	102.2	892.40
5	100.0	100.7	95.7	106.3	97.7	101.0	101.7	101.0	100.7	94.0	103.0	96.3	97.0	895.48
6	100.0	100.0	99.4	101.6	100.4	104.5	106.4	99.7	99.1	100.7	101.0	95.0	98.8	902.65
7	100.0	96.6	97.8	98.4	98.7	96.6	95.7	95.1	96.0	96.0	95.4	95.7	100.3	869.18
8	100.0	100.0	98.8	96.5	97.6	96.8	94.7	95.0	96.2	98.2	98.8	98.8	98.8	877.28
mean	100.0	99.0	98.8	100.0	98.9	97.5	99.2	98.7	100.1	98.6	99.4	98.2	100.5	891.67
%CV	0.03	2.07	1.93	3.3	2.99	5.12	5.68	2.53	3.65	3.05	3.02	3.36	2.53	1.69
SEM	0.01	0.72	0.67	1.17	1.05	1.76	1.99	0.88	1.29	1.07	1.06	1.17	0.90	5.32

The % calcium plasma level relative to the initial point following nasal administration of 300 units sCT.

( with 0.5% CS G)

subject	Times													AUC(0-9hr)
no.	0	0.5	1	1.5	2	2.5	3	4	5	6	7	8	9	
1	100.0	96.8	96.3	96.5	96.0	93.4	94.5	95.1	96.3	95.1	93.1	96.5	95.4	859.18
2	100.0	98.8	98.4	96.9	100.0	98.1	100.6	99.7	92.2	95.9	96.6	97.8	98.4	877.95
3	100.0	95.6	76.3	90.8	92.0	93.8	93.8	92.0	79.5	65.3	74.2	92.6	93.5	769.95
4	100.0	93.5	95.8	94.9	95.5	87.0	85.2	95.2	98.2	92.6	93.8	94.7	96.7	845.10
5	100.0	100.0	100.0	98.3	97.3	99.0	93.3	95.3	97.0	93.9	99.0	99.3	101.0	877.28
6	100.0	100.0	92.3	90.4	96.3	94.7	96.9	96.9	88.9	88.3	90.1	90.8	93.2	836.13
7	100.0	97.6	94.6	100.0	91.8	95.8	96.1	94.9	93.4	91.5	94.3	81.2	93.1	838.83
8	100.0	98.2	99.1	102.4	98.5	96.4	97.9	99.1	85.6	93.1	92.8	91.9	92.8	854.63
mean	100.0	97.5	94.1	96.3	95.9	94.8	94.8	96.0	91.4	89.5	91.7	93.1	95.5	844.88
%CV	0.03	2.28	8.11	4.34	2.99	3.92	4.81	2.59	7	11.2	8.23	6.08	3.14	4.04
SEM	0.01	0.79	2.7	1.48	1.01	1.31	1.61	0.88	2.26	3.55	2.67	2	1.06	12.08

## VITA

Miss Arisara Muangkum was born on 24th April 1970 in Pitsanulok, Thailand. She graduated with the Bachelor of Pharmacy Degree from the Faculty of Pharmacy, Chiangmai University in 1994. After graduation, she worked at Kamppet Hospital, Kamppet, Thailand for two years, before entering the Master's Degree program in Department of Pharmacy at the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

