Table 1. Effect of a single dose (10 mg/kg, i.p.) of sanguinarine on body and liver weight

	Change in b.wt (%)	Liver weight (g/100 g b.wt)*
Control	+ 5.08	5.03 ± 0.12
Treated	− 7.22	3.75 ± 0.06**

* Values represent mean ± SD of five samples. ** Significantly different ($p < 0.05$) from corresponding control.

Table 2. Effect of sanguinarine on serum and liver microsomal enzymes

Treatment	SGPT (Karmen units)*	SGOT (Karmen units)*	Cyt. P-450 (nmoles/mg microsomal protein)*	Benzphetamine N-demethylase (nmoles HCHO/min/mg microsomal protein)*
Control	29 ± 4	163 ± 17	0.659 ± 0.01	2.87 ± 0.15
Sanguinarine	44 ± 8**	296 ± 32**	0.459 ± 0.05**	1.99 ± 0.21**

* Values represent mean ± SD of five samples. ** Significantly different ($p < 0.05$) from corresponding control.

Next, to assess the toxic effect of sanguinarine on liver, the activities of serum enzymes (SGPT and SGOT) and the hepatic microsomal enzymes were used as the indices of toxicity in these studies. As can be seen in table 2, a single dose of sanguinarine caused almost 50% and 100% increase in the activities of SGPT and SGOT, respectively, indicating sanguinarine-induced liver injury was substantial. According to Urbanek-Karlowska, the tests of liver microsomal enzyme activities as indicators of liver damage are more useful than those of blood serum[9]. Therefore, the concentration of cytochrome P-450 and the activity of benzphetamine N-demethylase in the liver microsomes from sanguinarine-treated animals were determined (table 2). The results of the determinations show that the alkaloid caused a significant loss of cytochrome P-450 and benzphetamine N-demethylase activity, confirming the evidence of liver injury provided by serum enzyme tests. The inhibition of liver microsomal enzymes and the subsequent liver damage may have occurred due to the binding of the alkaloid to cytochrome P-450[4].

The above noted biochemical data were further substantiated by histological changes in the liver tissues (fig. 2). Livers from rats treated with sanguinarine showed swollen hepatocytes with hydropic changes. The nuclei of the cells also showed swelling and degenerative changes, all leading to necrosis. These microscopic lesions correlate positively with the biochemical alterations, but the evidence is not sufficient to conclude that the two events are directly related although it is known that necrogenic toxicants such as carbon tetrachloride severely affect serum and microsomal enzymes. However, the studies provide an evidence of hepatotoxic potential of sanguinarine which is an important food contaminant responsible for severe outbreaks of epidemic dropsy in the tropics.

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1 Tandon, R.K., Singh, D.S., Arora, R.R., Lal, P., and Tandon, B.N., Am. J. clin. Nutr. *28* (1975) 883.
2 Wilcocks, C., and Manson-Bahr, P.E.C., (eds), Manson's Tropical Diseases, 17th edn. ELBS and Ballire Tindall, London 1972.
3 Whittle, J.A., Bissett, J.K., Straub, K.D., Doherty, J.E., and McConnell, J.R., Res. Commun. Biochem. Path. Pharmac. *29* (1980) 377.
4 Peeples, A., and Dalvi, R.R., J. appl. Toxic. *2* (1982) 300.
5 Dalvi, R.R., and Peeples, A., J. Pharm. Pharmac. *33* (1981) 51.
6 Dalvi, R.R., and Howell, C.D., Bull. envir. Contam. Toxic. *17* (1977) 225.
7 Dalvi, R.R., Hunter, A.L., and Neal, R.A., Chem.-Biol. Interact. *10* (1975) 349.
8 Tandon, R.K., Tandon, H.D., Nayak, N.C., and Tandon, B.N., Ind. J. med. Res. *64* (1976) 1064.
9 Urbanek-Karlowska, B., Rooz. Panstw. Zakl. High. *31* (1980) 453.

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The effects of acute and chronic morphine on regional distribution of cardiac output in brain

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Summary. Both acute and chronic administration of morphine resulted in an increase in the percent cardiac output received by brain. However, various brain regions were affected differently by the drug treatments. The greatest increases in percent cardiac output received after chronic administration of morphine occurred in pons and cerebellum, while the greatest increases after acute administration occurred in cortex and midbrain. The changes found are in contrast with earlier studies which suggest that morphine has no effect on cerebral blood flow.
Key words. Guinea pig brain; cardiac output; morphine; cerebral blood flow.

Opioids, including β-endorphin, enkephalins and morphine, have been shown to depress cardiovascular parameters, including heart rate and blood pressure[1], as well as inhibit the responsiveness of the cardiovascular baro- and chemoreceptor reflexes[2]. Evidence suggests that these responses are mediated through central nervous system (CNS) receptors. When naloxone, a potent opioid antagonist, is administered i.c.v., the doses which are necessary to reverse many of the cardiovascular opioid effects are as much as 100-fold lower than doses administered i.v.[3]. In addition, only the stereospecific (−) isomer of naloxone is effective in this reversal[4], suggesting a specific CNS receptor-mediated response.

Changes in regional cerebral blood flow (rCBF) have been shown to be correlated with changes in neuronal activity in specific brain regions[5] and with changes in regional metabolic activity[6]. Changes in neuronal activity have also been shown to correlate with the utilization of blood born energy substrates[7]. However, it has been assumed that morphine does not alter

cerebral blood flow[8], while morphine can have inhibitory or stimulatory effects on regional neuronal activity. Taking this latter observation into account with the aforementioned correlations and cardiovascular effects, we thought it important to reassess the effects of morphine on rCBF.

The first phase of this experiment was designed to assess the effects of chronic morphine treatment on the distribution of cardiac output to several brain regions. Male guinea pigs were anesthetized with 35 mg/kg sodium pentobarbital and surgically prepared by left ventricular cannulation via the right carotid artery. Unlike other species, the adult guinea pig receives the majority of its cerebral blood flow via vertebral arteries. Therefore, cannulation of the carotid artery will not adversely affect cerebral blood flow. This cannula was used for the injection of radioactively labeled microspheres[9]. This procedure allowed for the determination of the percent cardiac output (%CO) received by various brain regions[9]. Morphine sulphate pellets (65 mg/pellet; 1.5 pellets/100 g b.wt) were implanted s.c., approximately 2 h after surgery[10]. Just prior to and 48 h after pellet implantation, between 4×10^5 and 8×10^5 tracer microspheres were injected into the left ventricle[9]. This chronic morphine treatment resulted in a significant increase ($p < 0.05$)[11] over pre-drug treatment in the %CO received by the total brain (40.8% (table)), the greatest increase being in cerebellum (71.8%) and pons (60.9%) (fig. 1; table).

The second phase of the experiment was designed to assess the effects of an acute i.v. injection of morphine on the %CO received by the brain. Guinea pigs were surgically prepared as before with the addition of a jugular cannula for use as an i.v. injection site for a bolus of morphine. Animals were allowed to recover for 24 h before experiments were performed. A series of acute i.v. injections of morphine was then performed and brain morphine levels measured 15 min post-morphine[12]. It was found that 200 mg/kg morphine[13] produced a brain morphine level of 9.95 ± 2.35 µg/g tissue which was not significantly different from the chronic 48-h level (9.0 ± 2.1 µg morphine/g tissue). We therefore used this dose to compare the effects of acute morphine treatment with those of chronic treatment on the %CO received by brain regions.

The pre-morphine distribution values for both chronic and acute treatment were not significantly different. This indicates that the anesthesia had no measureable effect on pre-morphine distribution values in either group. Acute morphine administration resulted in a significant increase (66.5%) in %CO to brain at 15 min post-morphine (table), which was not significantly different[11] from the increase observed in the chronically-treated animals at 48 h. In contrast to the chronic study, the regions with the largest increase in %CO received after acute morphine were midbrain (77.3%) and cortex (74.9%) (fig. 2; table).

All changes in %CO occurred in the absence of any significant change in the mean left ventricular pressure at the time periods studied[14]. This is consistent with previous findings demonstrating only transient effects of morphine on blood pressure[15]. Others have reported opiate receptor-mediated changes in left ventricular pressure development over time[16], an index of myocardial contractility, which may alter cardiac output and, therefore, alter the absolute rCBF, despite unaltered pressure or heart rate. However, many consider this index to be a poor indicator of contractibility. There is no direct evidence to indicate that cardiac output would be significantly altered after morphine injection at the time measurements made in the present study. In addition, the regional differences in magnitude of change in %CO in our study (i.e. 14.8% change in hypothalamus vs 71.8% change in cerebellum at 48 h post-morphine; table) affirm changes in rCBF, regardless of absolute rCBF values, since an alteration in CO alone would result in uniform regional distribution changes.

These findings indicate that the current assumption concerning the lack of an effect of morphine on rCBF is incorrect. In both treatment protocols, which result in similar brain morphine levels, the %CO received by brain increases. However, the two treatments used in this study (chronic and acute) produced different patterns of changes in specific brain regions. Since adaptation to the prolonged presence of morphine (tolerance) re-

Percent increase in regional distribution of cardiac output

Brain area	Acute*	Chronic**
Total brain	66.52	40.81
Medulla	51.73	34.03
Pons	38.54	60.85
Cerebellum	50.79	71.78
Hypothalamus	40.47	14.83
Midbrain	77.31	39.99
Cortex	74.90	35.27

*Change from pre-morphine treatment to 15 min post-morphine treatment. **Change from pre-morphine treatment to 48 h post-morphine treatment initiation.

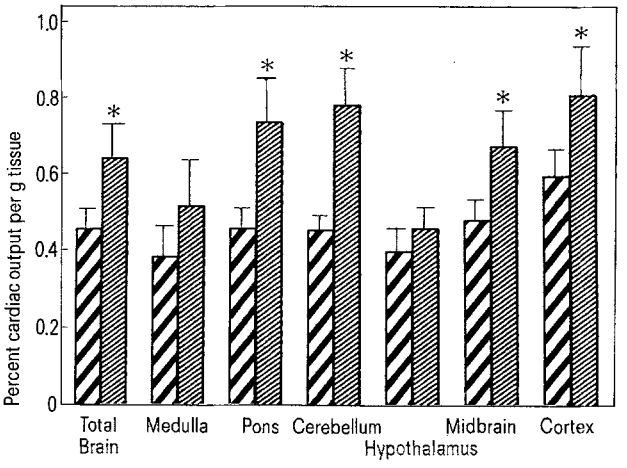

Figure 1. Measurement of %CO received by various brain regions before morphine pellet implantation (▨), and 48 h after chronic exposure to morphine (▨). Values represent means ± SEM. Asterisks denote significant difference from pre-drug treatment at $p < 0.05$ using paired t-test ($n = 7$).

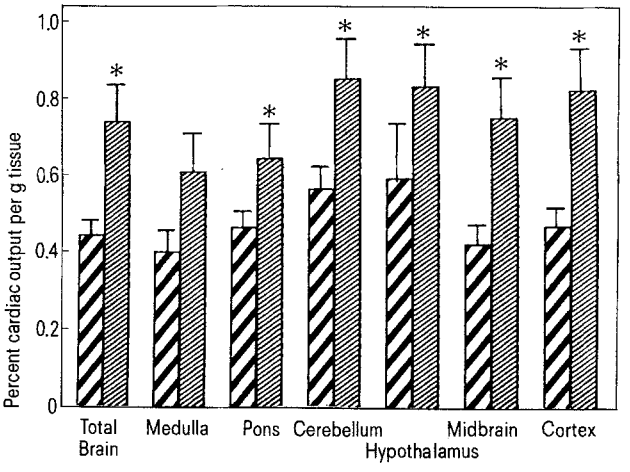

Figure 2. Measurement of %CO received by various brain regions before (▨), and 15 min post 200 mg/kg morphine, i.v. (▨). Values represent means ± SEM. Asterisks denote significant difference from pre-drug treatment at $p < 0.05$ using paired t-test ($n = 7$).

sults in changes in central nervous system activity, these treatment related regional patterns may represent one aspect of this adaptation process, wherein greater increases in blood flow compensate for commensurate increases in regional neuronal activity.

The distribution of a population of opioid receptors in the brain has been delineated in a number of species, including the rat, using dihydromorphine[17]. One region with a high density of these opioid receptors in the rat is the midbrain. Another region with a relatively dense opioid receptor population is the cortex. Interestingly, we found that these regions underwent the greatest increase in %CO at 15 min post-200 mg/kg, i.v., morphine. In contrast, those regions which underwent the greatest increase in %CO 48 h after continuous morphine administration, the pons and cerebellum, are regions which tend to have a more sparse opioid receptor population in the rat. Recently, a number of different opioid receptors have been identified in brain. It would be of interest to compare the density of these receptors in various brain regions with changes in blood flow using appropriate agonists to those receptors.

Finally, the present data suggests that both acute and chronic morphine administration alters rCBF, in contrast to earlier statements suggesting no change following morphine treatment[8]. Therefore, further studies are needed to characterize the mechanisms by which opioids affect blood flow to brain and regional cerebral blood flow.

1 Laubie, M., and Schmitt, H., Eur. J. Pharmac. *71* (1981) 401; Fennessy, M.R., and Rattray, J.F., Eur. J. Pharmac. *14* (1971) 1; Moore, R.H., and Dowling, D.A., Reg. Peptide *1* (1980) 77; LeMaire, I., Tseng, R., and LeMaire, S., Proc. natn. Acad. Sci. USA *75* (1978) 6240.

2 Yukimura, T., Stock, G., Stumpt, H., Unger, T., and Ganten, D., Hypertension *3* (1981) 528; McQueen, D.S., and Ribeiro, J.A., Br. J. Pharmac. *71* (1980) 297.

3 Holaday, J.W., and Faden, A.I., Brain Res. *189* (1980) 295.

4 Faden, A.I., and Holaday, J.W., J. Pharmac. exp. Ther. *212* (1980) 441.

5 Olesen, J., Brain *94* (1971) 635; Kato, M., Ueno, H., and Black, P., Exp. Neurol. *42* (1974) 65.

6 Kontos, H.A., Rasper, A.J., and Patternson, J.L., Stroke *8* (1977) 358; Wahl, M., and Kuschinsky, W., Pflügers Arch. *362* (1976) 55.

7 Kennedy, C., Des Rosiers, M.H., Jehle, J.W., Reivich, M., Sharpe, F., and Sokoloff, L., Science *187* (1975) 850; Sokoloff, L., J. Neurochem. *28* (1977) 897.

8 Jaffe, J.H., and Martin, W.R., in: The Pharmacological Basis of Therapeutics, 5th edn, chap.15. Eds L.S. Goodman and A. Gilman. Macmillan Publishing Co., New York 1975.

9 Each set of microspheres (15 ± 1 μm) was labelled with one of three isotopes: ^{46}Sc, ^{85}Sr or ^{141}Ce. Distribution of cardiac output was calculated according to the methods of Ferguson, J.L., Spitzer, J.J., and Miller, H.I., J. surg. Res. *25* (1978) 236.

10 McGinity, J., and Mehta, C., Pharmac. Biochem. Behav. *9* (1978) 705.

11 Two-way ANOVA between tissue and time indicated a significant difference between pre- and post-treatment times in both acute and chronic treatment. Individual differences between time in each tissue were tested using paired t-test. The difference between the total brain percent change in chronic and acute treatments was tested for significance with Student's t-test after arcsin transformation of the data.

12 Kupferberg, H., Bukhalter, A., and Way, E., J. Pharmac. exp. Ther. *145* (1964) 247.

13 Although 200 mg/kg morphine is higher than doses administered to many species, in pilot studies with guinea pigs, up to 400 mg/kg was administered before any mortality was observed. No seizures were observed in guinea pigs which received 200 mg/kg morphine i.v.

14 Mean left ventricular blood pressures:

	Acute	Chronic
Control	49.6 ± 1.47	57.4 ± 1.93
Post-morphine	48.8 ± 1.62	52.9 ± 2.12

15 Evans, A.G.J., Nasmyth, P.A., and Stewart, H.C., Br. J. Pharmac. *7* (1952) 542; Fennessy, M.R., and Rattray, J.F., Eur. J. Pharmac. *14* (1971) 1.

16 Vargish, T., Reynolds, D.G., Gurll, N.J., Lechner, R.B., Holaday, J.W., and Faden, A.I., Circ. Shock *7* (1980) 31; Reynolds, D.G., Gurll, N.J., Vargish, T., Lechner, R.B., Faden, A.I., and Holaday, J.W., Circ. Shock *7* (1980) 39.

17 Wolozin, B.L., Nishimura, S., and Pasternak, G.W., J. Neurosci. *2* (1982) 708.

18 This work was supported by Chicago Heart Association grant No. C82-13 and BRSG 82508.

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The effect of cyproheptadine on insulin biosynthesis

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Summary. The effect of a potent antiserotonin-antihistaminic compound, cyproheptadine (CPH) on insulin biosynthesis was studied in pancreatic islets isolated from CPH-treated rats. Though insulin content of islets was markedly reduced in CPH-treated rats, the incorporation of radiolabeled leucine into proinsulin and insulin fractions was not affected with respect to the rate and amount. It is concluded that CPH may deplete insulin content of the islets through causing the leakage of insulin.
Key words. Pancreatic islets, rat; insulin biosynthesis; cyproheptadine.

Cyproheptadine (CPH), a potent antiserotonin-antihistaminic compound, has been reported to cause glucose intolerance in rats when given orally in large dose[3,4]. The morphological investigation of these rats revealed selective abnormalities of pancreatic beta cells such as degranulation and vacuolization of rough endoplasmic reticulum. In addition CPH caused a remarkable reduction of insulin content of pancreatic islets in a few days of the treatment[5]. These results suggest the specific effect of this compound on pancreatic beta cells. Though there have been reports indicating the inhibitory action of CPH on insulin secretion[6,7], its effect on biosynthetic process of insulin has not been clarified yet. The present study was conducted to investigate the effect of CPH on insulin biosynthesis using pancreatic islets isolated from CPH-treated rats.