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i.r. β-Endorphin, Corticosterone, Cholesterol and Triglyceride Concentrations in Rat Plasma After Stress, Cingulotomy or Both

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RECHER, H., G. L. WILLIS, G. C. SMITH AND D. L. COPOLOV. *i.r. β-endorphin, corticosterone, cholesterol and triglyceride concentrations in rat plasma after stress, cingulotomy or both.* PHARMACOL BIOCHEM BEHAV 31(1) 75–79, 1988.—Plasma i.r. β-endorphin, corticosterone, cholesterol and triglyceride concentrations were determined in male Sprague-Dawley rats after exposure to running or swimming stressors or after surgical ablation of the cingulum bundle. While cingulotomy alone altered only the plasma triglyceride concentrations, the combination of cingulotomy plus running stress significantly increased plasma i.r. β-endorphin and triglyceride concentrations above those seen in animals receiving only a running stress. Triglyceride concentrations in cingulotomy plus swimming stress were significantly elevated above those in animals receiving a severe stress only. While the exposure to running and swimming increased plasma β-endorphin significantly above control levels, plasma corticosterone was not affected by these stressors. Changes in plasma cholesterol and triglycerides were also differentially affected by cingulotomy or stress exposure. These results indicate that various stress hormones are affected differentially by exposure to various experimental procedures which are employed as stressors.

i.r. β-Endorphin Corticosterone Triglycerides Cholesterol Cingulotomy Stress hormones

β-ENDORPHIN (βEP) and corticosterone (CST) are two substances which are released into the blood when an organism undergoes stress. While it is well known that control over this response is mediated, at least in part, by the catecholaminergic (CA) innervation of the basomedial hypothalamus (BMH) [1, 11, 19, 20], there are other brain areas, far removed from the BMH, which may also participate in the control of βEP release. In a recent pilot study [3] we have examined the relationship between surgical ablation of the cingulum in normal and morphine dependent rats and plasma βEP concentrations, since it has been suggested that cingulate cortical lesions can alleviate morphine withdrawal in animals and man [4,8]. Although lesions of the cingulum bundle did not cause elevation of plasma βEP concentrations or attenuation of morphine withdrawal in most animals, there was a tendency for this to occur in a few of the animals tested [3]. In consideration of the importance of the cingulum in morphine addiction and that stress and morphine dependence are associated with pituitary function [3], further experiments on the effects of cingular lesions during various forms of stress were studied.

In the present study we have examined the effect of bilateral cingulotomy on plasma immunoreactive (i.r.) βEP and CST in animals undergoing swimming and running stress. We hypothesized that the elevation in plasma βEP concentrations observed previously in a few of the cingulotomized rats [3] would be increased further when these animals underwent stress. In addition, we have measured plasma triglyceride and cholesterol concentrations since they too can vary in response to the application of various physical and psychological stressors [2, 11, 16, 17, 21]. Such parameters may be of considerable importance in stress related disorders such as coronary disease [6, 18, 23].

METHOD

Eighty male Sprague-Dawley rats weighing approximately 200 g at the time of surgery were used in this study. They were group housed in plastic boxes with wire tops. The animals were allowed ad lib access to rat cubes (Clarke King, Melbourne) and tap water for the duration of the experiment. The boxes were stored in racks in a room where temperature

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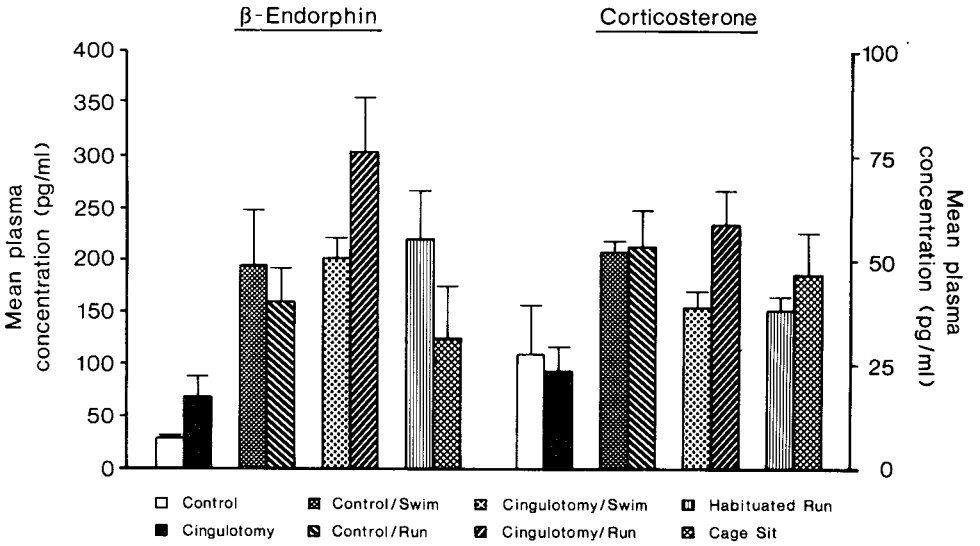

FIG. 1. Plasma i.r. β-endorphin or corticosterone concentrations in rats undergoing sham operations, cingulotomy, running stress, swimming stress, habituated running or exposure to the running wheel. The T-bars represent the standard error of the mean.

was controlled at 22°C(±2°C) and a 12 hr light/dark cycle was maintained with lights on at 0700 hr.

Surgery

Thirty of the animals were anaesthetised with 84 mg/kg of Alfathesin (Glaxo Pharmaceuticals; Port Fairy, Australia) and then placed in a stereotaxic instrument. Each of these animals received 2 pairs of bilateral, radiofrequency lesions of the cingulum bundle (50°C for 60 sec). The coordinates for the lesions were: Anterior site: A+1.2 mm, L=±1.5 mm, D=−2.0 mm; Posterior site: A=−0.8 mm, L=±1.5 mm, D=−1.9 mm. The 50 remaining animals were anaesthetised, an incision was made, the periosteum removed, the skull drilled and the incision was closed; but no lesion was made.

Procedure

At 21 days after surgery, a time chosen to allow animals to recover from the stress of surgery [3], animals were randomly allocated to the following groups: 10 animals which received sham surgery served as controls. Ten of the cingulotomised animals were placed in a motorised running wheel [9, 10, 15] running at 8 rpm, for 10 minutes. Ten other cingulotomised rats were placed in a 25×60×130 cm liquid chromatography tank filled with water (22°C) and allowed to swim for a 10 minute period [19]. Two other groups of 10 sham operated animals were also allowed to swim and run as described above. To determine the effects of physical exercise on the parameters studied, 10 of the remaining sham operated controls were exposed to habitual running by placing them in the running wheel every day for 5 days, and were forced to run 10 minutes each day. To determine the stress of the novel environment of the running wheel the remaining 10 sham operated animals were placed in the running wheel without turning it on. Finally, 10 cingulotomised animals were sacrificed without being exposed to any of the stressors. All animals were sacrificed by decapitation immediately following exposure to stress. Trunk blood was collected in heparinised tubes and then centrifuged at 2,500 rpm. The plasma was then divided into aliquots and stored at −70°C for subsequent i.r. βEP [12], corticosterone [13], triglyceride (Human Gesellschaft fur Biochemica und Diagnostica) and cholesterol assay (Boehringer Mannheim Diagnostica).

The brains of cingulotomised animals were stored in 10% buffered formaldehyde solution after sacrifice and then sectioned at 50 μm and the lesion damage resulting from cingulotomy plotted on plates from Pelligrino *et al.* [14]. All data were analysed with a Canon BX-10 statistical package.

RESULTS

As shown in Fig. 1, cingulotomy failed to produce a significant increase in plasma i.r. βEP concentrations as compared to sham operated controls. In sham operated animals the running and swimming stress caused a significant increase in plasma i.r. βEP concentrations as compared to sham operated controls [two-way ANOVA, $F(2,62)=24.5$, $p<0.001$]. Plasma i.r. βEP concentrations in cingulotomised animals undergoing running stress were significantly greater than those in running stressed,sham operated controls [two-way ANOVA, $F(2,62)=3.4$, $p<0.05$: post hoc Mann Whitney U-test, $U=22.0$, $p<0.05$]. Plasma i.r. βEP concentrations were not significantly different in cingulotomised versus sham operated animals undergoing swim stress. The plasma i.r. βEP concentrations of animals exposed to habitual running and to exposure to the cage without running were significantly elevated above those of sham operated controls (Student's *t*-tests: $p<0.001$ and $p<0.05$, respectively).

As shown in Fig. 1, CST concentrations were not significantly different in cingulotimised, sham operated swim or running stressed, habituated running stressed or running cage exposed animals in comparison to sham operated controls. In the cingulotomised animals, only those undergoing running stress showed a significant elevation in plasma CST concentration [two-way ANOVA, $F(2,62)=8.1$, $p<0.001$: post hoc Mann Whitney U-test, $U=99.0$, $p<0.05$].

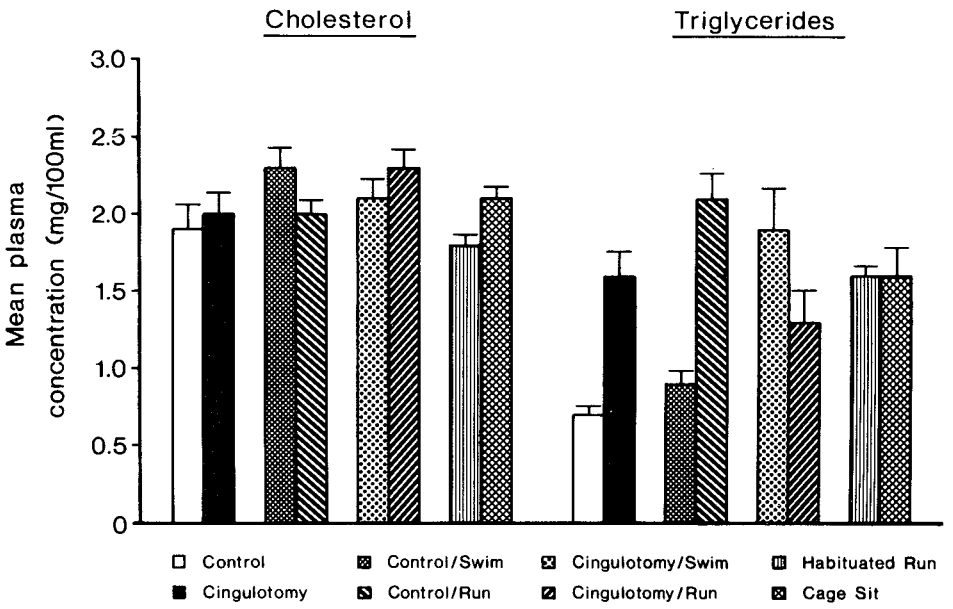

FIG. 2. Plasma cholesterol and triglyceride concentrations in rats undergoing sham operations, cingulotomy, running stress, swimming stress, habituated running or exposure to the running wheel. The T-bars represent the standard error of the mean.

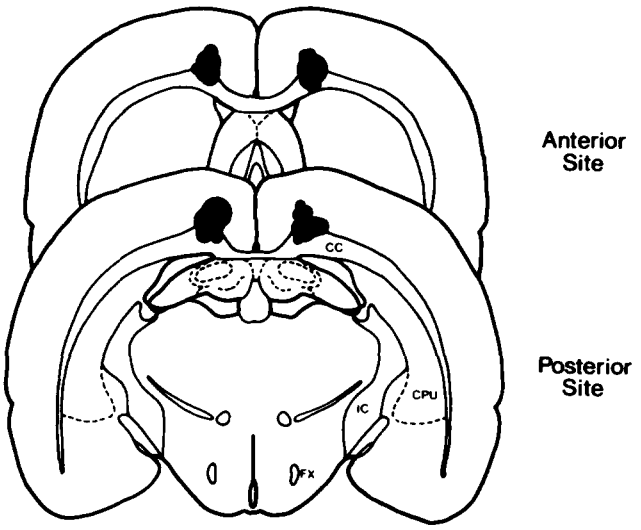

FIG. 3. Representative histological plates [14] depicting the lesion damage resulting from radio frequency lesions (50°C for 60 sec) of the anterior and posterior cingulum bundle. Each animal received bilateral lesions at both sites. (cc=corpus callosum, ic=internal capsule, cpu=caudate putamen, fx=fornix.)

The plasma cholesterol concentrations of all groups were similar and no significant differences after the various treatments were detected (Fig. 2).

Plasma triglyceride concentrations (Fig. 2) after running stress or swimming stress, were significantly elevated [two-way ANOVA, $F(2,62)=3.8, p<0.05$]. Cingulotomy also caused a significant elevation of plasma triglyceride concentrations [two-way ANOVA, $F(2,62)=4.01$, $p<0.05$] in the unstressed, run-stressed and swim-stressed animals (respectively, post hoc Mann Whitney U-test, $U=0.0$, $p<0.001$; $U=22.5$, $p<0.05$; $U=106.0$, $p<0.001$). Habituated running and cage sitting caused significant elevations in plasma triglycerides as compared to sham operated controls (Student's t-test, $p<0.01$ in both cases).

As shown in Fig. 3, the lesions resulting from cingulotomy were symmetrical and were usually located in close proximity to the cingulum bundle. Occasionally a minimal amount of damage resulting from the lesions extended dorsal into the cerebral cortex and ventral into the corpus callosum.

DISCUSSION

The present results indicate that while cingulotomy alone does not cause a significant elevation of plasma i.r. βEP concentrations, this procedure can facilitate the rise in plasma concentrations of this opioid which are seen after the application of a running stress. That such an effect is not observed when cingulotomy is combined with other stressors, such as swimming, may indicate that all stressors may not result in the same hormonal profiles. Such an effect could be useful in developing a subclassification system for different stressors. It has been suggested that βEP, adrenocorticotropic hormone and CST release in animals and man all function in parallel in response to a physical stressor [5,21]. However, we and others have found previously [1,19] that CST release is not always influenced in the same way as is βEP release in response to a swim stress. This finding was confirmed in the present study in that cingulotomy or stress-induced changes in plasma CST concentrations were not observed in spite of the fact that plasma i.r. βEP was altered in response to the various surgical and stress treatments.

One endeavour of the present experiment was to study the effects of a chronic physical stressor (habituated wheel

running) in comparison to acute physical (swimming or running) or physiological method (cingulotomy) for initiating a neuroendocrine stress reaction. Forced running of a short duration (5, 15, 60 min) is reported to elevate CST concentrations in plasma [7,10] and there appears to be a fairly direct (linear) relationship between CST concentrations and duration of stressor [9]. In contrast, prolonged voluntary running can result in attenuation of acute stress-induced plasma CST elevation and may be regarded as an organism's way of exerting control over a potentially stressful event [21]. While this may explain why we did not observe elevated CST levels in the animals exposed to habituated running it does not explain why the other, more acute stressors, were without effect.

The finding in the present paper that cingulotomy does not cause a significant increase in i.r. βEP concentrations is in disagreement with our previously reported findings [3]. In view of previous clinical [8] and experimental [4] reports which describe the importance of the cingulum in the morphine abstinence syndrome, we hypothesised that plasma i.r. βEP concentrations would serve as an index as to how endogenous opiate systems were modified under the conditions of morphine addiction, morphine abstinence and the alleviation of morphine craving and withdrawal by cingulotomy. While the effect of cingulotomy on i.r. βEP is only minor, such an effect becomes detectable when select stressors are applied in combination with this surgical procedure. We therefore conclude that cingulotomy may alleviate the morphine abstinence syndrome by modifying the function of endogenous opiate systems and that care must be exercised in selecting the proper conditions under which to detect such modified function.

It has been reported previously that changes in plasma cholesterol concentrations will permit the discrimination between varying degrees of exposure to psychological stressors, even when such periods of exposure were less than 1 hour [1]. Plasma triglycerides are reported to reciprocate those of cholesterol and when triglycerides are increased cholesterol concentrations are decreased. However, in the present study, we did not detect such a relationship between these two parameters. But we did observe an increase in triglyceride concentrations, with the exception of the running and swimming stress, and this is in disagreement with previous reports which describe reduced plasma concentrations of stress hormones in rats receiving acute shock [22] or long-term exposure to other stressful stimuli [9]. While there is only a limited amount of literature dealing with the problem of the mechanisms involved in the control of lipid metabolism in plasma, it has been suggested that such factors are important in the metabolic control of digestive states [2]. The present results indicate that the brain (via the cingulum) as well as the type and duration of stressor may play an important role in the regulation of lipid function and hormonal responses in an organism undergoing stress.

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