行政院國家科學委員會補助專題研究計畫成果報告

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計畫類別：☐個別型計畫　　☐整合型計畫

計畫編號：NSC89－2320－B－040－003－

執行期間：88 年 8 月 1 日至 89 年 7 月 31 日

計畫主持人：吳文俊

共同主持人：

本成果報告包括以下應繳交之附件：

☐赴國外出差或研習心得報告一份

☐赴大陸地區出差或研習心得報告一份

☐出席國際學術會議心得報告及發表之論文各一份

☐國際合作研究計畫國外研究報告書一份

執行單位：私立中山醫學院

中　華　民　國　八十九年　十月　二十七日

行政院國家科學委員會專題研究計畫成果報告

探討類皮質醣和其他緊迫性內分泌物質的總濃度與免疫功能的關係

Evaluate the relationship between the total concentrations of glucocorticoids and the other stress related neuroendocrine mediators and immune functions

計畫編號：NSC 89-2320-B-040-003
執行期限：88 年 8 月 1 日至 89 年 7 月 31 日
執行機構及單位名稱：中山醫學院 毒理學研究所
主持人：吳文俊

一、中文摘要

當身體受到緊迫時，類皮質醣和其他神經內分泌物質的釋放量會增加，這些內分泌物質的過度釋放可造成免疫功能的下降。許多和環境有關的化學物質在實驗動物的研究上可增加血中類皮質醣的濃度。這種情形也發生在人類上。然而免疫功能受到抑制的程度與類皮質醣濃度之間的關係並不清楚。目前的研究大多著重高濃度的類皮質醣與免疫功能的關係。各種化學物質產生類皮質醣的濃度與總量不一，其影響免疫功能的程度也就難以預測。如果有一種線性迴歸的模式可預測類皮質醣的總濃度和免疫功能的關係，則這種模式可被用來預測是否由任一化學物質所誘發的類皮質醣可降低免疫功能。我們的實驗數據顯示類皮質醣的總濃度（不同時間下所測得的濃度總和）而非最高濃度與 MHC II 蛋白表達及自然殺手細胞功能下降呈現線性關係。因此，本計畫所發展的線性迴歸模式可預測類皮質醣造成某些免疫指標的下降與其總濃度有密切關係。其他緊迫內分泌物質則可加強類皮質醣對某免疫功能的抑制作用。

Abstract

The physiological response to stressors can be immunosuppressive, and glucocorticoids and other neuroendocrine mediators have been implicated. Many environmentally relevant chemicals increase glucocorticoid levels in the rodent models commonly used to evaluate these chemicals. Elevated glucocorticoid levels have also been observed in humans exposed to environmental toxicants. This raises questions about the amount and type of immunosuppression that can be expected following a particular increase in glucocorticoid concentrations in the blood. At present, such questions can not be answered except by evaluating each chemical individually. If predictive regression models relating glucocorticoids and immunosuppression could be developed, they could be used whenever there was reason to suspect that a chemical was inducing a stress response sufficient to suppress the immune system. This study was designed to develop regression models to predict the relationship between the area under the corticosterone concentration vs. time curve (AUC) and expression of MHC II molecules and natural killer (NK) cell activity. Models were developed using mice treated with exogenous corticosterone and mice subjected to various periods of restraint stress. Models relating corticosterone AUC to MHC II expression and NK cell activity was reversely proportional, whether the corticosterone was exogenous or produced as part of a restraint stress response.

二、緣由與目的

A number of chemicals can induce neuroendocrine stress responses in rodent models, as indicated by elevated

corticosterone concentrations. In some cases a stress response is only noted near the maximum tolerated dose (Kunimatsu et al., 1996). The stress response induced by some of these compounds suppresses spleen or thymus cellularity, but not selected functional immunological parameters (Burns et al., 1994; De Krey et al., 1993). Using antagonists and other approaches, it has been shown that some compounds mediate functional immunosuppression primarily via upregulation of neuroendocrine mediators (Weiss et al., 1996; Wu and Pruett, 1997). However, it should be emphasized that only a small percentage of immunotoxicants have been evaluated for induction of stress responses (Pruett et al., 1993), and few comprehensive studies have been done to definitively demonstrate the role of a particular neuroendocrine mdiator in immunosuppression.

The biological effects of stress-induced neuroendocrine on both the degree and duration of the increases in their concentration. For glucocorticoids, this may best be expressed as the area under the concentration vs. time curve (AUC), and this value usually correlates strongly with biological effect (Lew et al., 1993). However, previous studies examining the correlation between neuroendocrine mediators and suppression of immunological parameters have not utilized corticosterone AUC values to develop predictive models. Determining the quantitative relationship between glucocorticoid exposure and immunosuppression is not only essential for a full understanding of stress-induced immunosuppression, but it has practical implications as well.

三、結果

Area under the corticosterone concentration vs. time curve (AUC). The graphs shown in Figure 1 are the same as the ones used to calculate AUC, except that the symbols and error bars were omitted from the scanned images. The AUC value derived from each graph is noted, and it is clear that administration of vehicle once or twice increases the corticosterone AUC and that exogenous corticosterone dose-responsively increases the AUC values. The AUC values also increase progressively with increasing duration of restraint. It should be noted that peak corticosterone levels are similar when corticosterone at 18 mg/kg is administered once (1X) or twice (at 0 and 2 h, designated 2X), but the AUC is considerably greater for mice given 2 doses. AUC values also increase with increasing duration of restraint, and the peak corticosterone levels were comparable in mice restrained for 6 or 8 h and in mice treated with corticosterone at 18 mg/kg.

Regression analysis of corticosterone AUC and suppression of surface MHC class II on splenocytes. Figure 2 illustrates the relationship between corticosterone AUC and the expression of MHC class II protein on splenocytes in mice treated with exogenous corticosterone or subjected to restraint stress. The AUC values for each dosage of corticosterone or period of restraint were obtained from the data illustrated in Figure 1, and these were plotted against data on MHC class II expression obtained using the same experimental conditions. The data are expressed as the percent of naïve control MHC class II levels transformed to the natural log (natural log values of 2.5-5.0, as shown in Figure 2, correspond to 12.2-148.4% of control). The regression lines and the 83.7% confidence intervals are shown. The 83.7% confidence intervals overlap over the entire range covered by the data from this study. The AUC values and 95% confidence intervals required to produce 50% inhibition of MHC class II expression were: 3491 ng/ml/h for exogenous corticosterone and 2911 ng/ml/h for restraint stress.

Regression analysis of corticosterone AUC and suppression of splenic NK cell activity. Figure 3 illustrates the relationship between corticosterone AUC and splenic NK cell activity (expressed as percent of naïve control) in mice treated with exogenous corticosterone or subjected to restraint stress. The regression lines and 83.7% confidence

intervals are shown. The confidence intervals overlap, indicating that there is no significant different between the corticosterone AUC vs. NK cell activity relationship depending on whether the corticosterone was derived exogenously or as part of a restraint stress response. The AUC values and 95% confidence intervals required to produce 50% inhibition NK cell activity were: 8585 ng/ml/h for exogenous corticosterone and 8170 ng/ml/h for restraint stress.

四、討論

Results shown here indicate that there is a significant linear relationship between corticosterone AUC and suppression of MHC class II expression or suppression of NK cell activity.

Restraint stress induces a number of neuroendocrine mediators that can have immunosuppressive effects, including corticosterone, catecholamines, and endogenous opioids (Ader et al., 1990). In particular, catecholamines remain elevated in mice during restraint sessions for as long as 16 h (Komori et al., 1996). The response to restraint stress in mice involves increased concentration of corticosterone and catecholamines throughout the restraint period. It is expected that immunological parameters affected primarily by corticosterone would be comparably suppressed at similar corticosterone AUC values by exogenous corticosterone and by restraint stress. In contrast, parameters affected substantially by stress-induced mediators other than corticosterone should be suppressed more by restraint than by exogenous corticosterone at equivalent corticosterone AUC values. Thus, results from the present study suggest that both MHC class II expression and splenic NK cell activity are suppressed primarily by corticosterone, at least under the conditions used here.

五、計畫成果自評

Predictive linear models should be applicable for animal to human extrapolation. Available data from human and laboratory animals suggest that the effects of similar stressors on the immune system are comparable in rodents and humans. By administering cortisol to humans, then measuring immunological parameters, it should be possible to develop models similar to those developed in mice. The mathematical relationship between the mouse and human models should allow direct extrapolation of data obtained in mice to expected results in humans.

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Figure 1

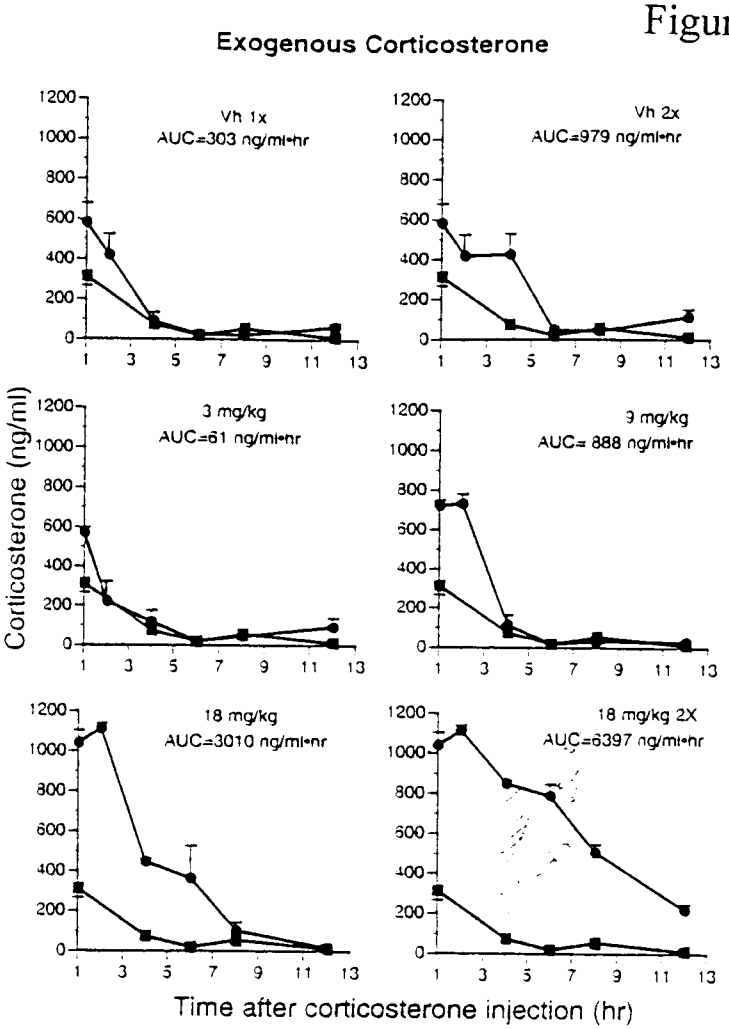
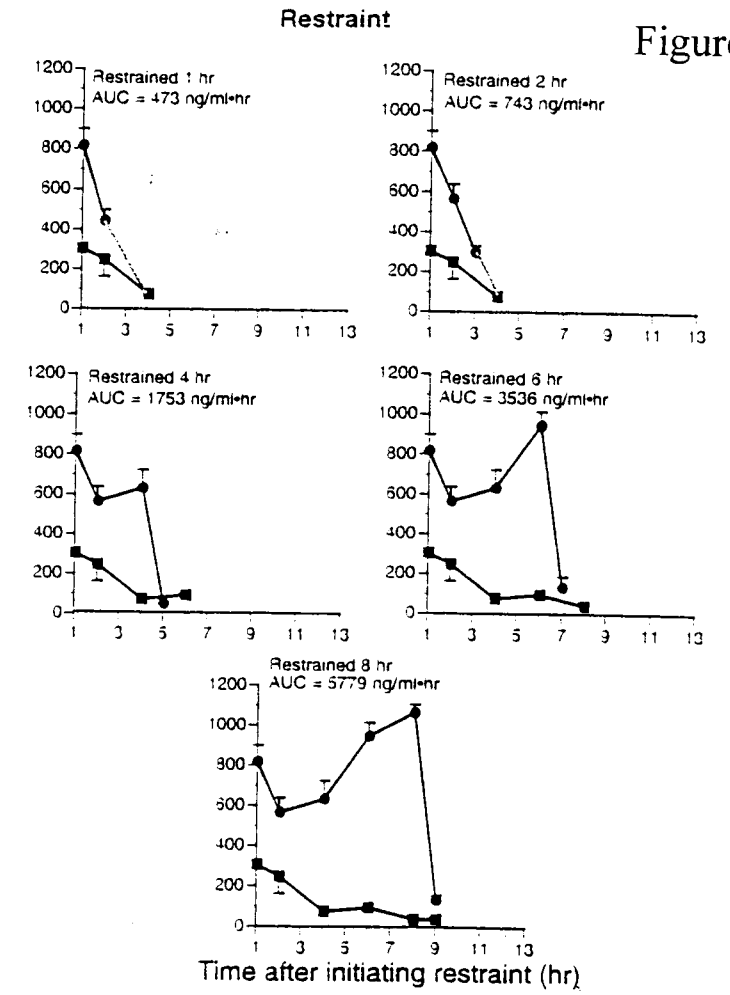


Figure 1



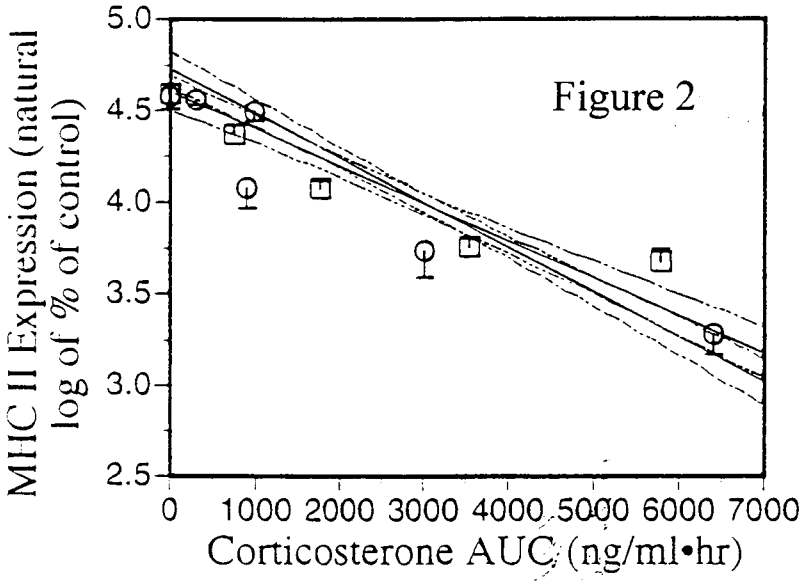


Figure 2

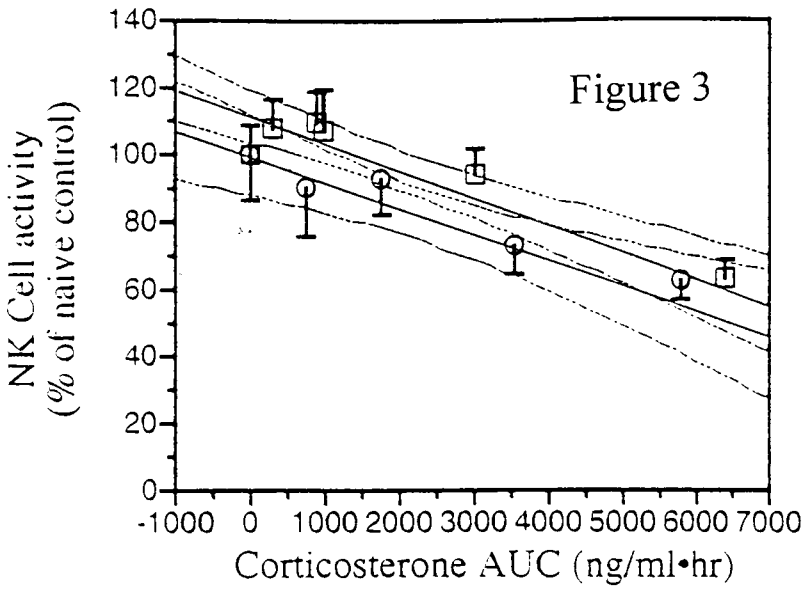


Figure 3