EFFECT OF TWO PROSTAGLANDIN SYNTHESIS INHIBITORS, INDOMETHACIN AND ACETYLSALICYLIC ACID, ON PLASMA ACTH AND CORTISOL LEVELS IN MAN

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ABSTRACT

The aim of this study was to investigate the possible role of prostaglandins (PG) in the control of the hypothalamic-pituitary-adrenocortical axis in normal volunteers. Acute oral administration of 100 mg indomethacin (ID) or 1.5 g acetylsalicylic acid (ASA) did not alter ACTH and cortisol plasma levels. Administration of 300 mg daily ID for 4 days delayed the onset, but increased the magnitude, of the response of ACTH to insulin hypoglycaemia, while it blunted the cortisol response. Administration of 3.2 g ASA daily depressed ACTH response to hypoglycaemia leaving the cortisol response unchanged, except for a 15 min delay in onset. These results are interpreted assuming that ID and ASA chiefly acted at the pituitary and hypothalamic level, respectively, and that ID, but not ASA, interfered with adrenocortical cortisol production. Our findings support the concept, based on animal studies, that PG enhance hypothalamic CRF release and adrenocortical steroidogenesis and may restrain ACTH secretion in the pituitary.

Several lines of evidence suggest that prostaglandins (PG) may be involved in the physiological regulation of the hypothalamic-pituitary-adrenocortical axis (De Wied et al. 1969; Peng et al. 1970; Hedge 1976, 1977). However, many aspects of this putative function of PG, such as the nature – stimulating or...
inhibiting – of their effect(s) or their site(s) of action. have not been defined. The available data favour the concept that PG enhance ACTH secretion by promoting the release of corticotrophin-releasing factor (CRF) from the hypotalamus (De Wied et al. 1969; Peng et al. 1970; Hedge 1976; Hedge & Hanson 1972). However, recent studies have also raised the possibility that PG restrain in the pituitary, the ACTH release stimulated by CRF (Hedge 1976; Hedge & Thompson 1975). Finally, a direct favouring effect of PG on adrenocortical steroidogenesis has been demonstrated (Flack et al. 1969; Flack & Ranwell 1972; Saruta & Kaplan 1972; Gallant & Brownie 1973; Warner & Rubin 1975; Spät & Jázan 1975). Present knowledge of PG influence on the hypothalamic-pituitary-adrenocortical function is largely derived from animal studies, in most of which pharmacological doses of PG have been administered locally or systemically.

To obtain additional information on this topic, we have studied the effects of acute and protracted administration of indomethacin (ID) and acetylsalicylic acid (ASA), two inhibitors of PG synthesis, on basal and insulin-stimulated plasma ACTH and cortisol levels in normal subjects.

PATIENTS AND METHODS

Forty female subjects, aged 19–56 years, mean 34.6 years, gave an oral informed consent to participate in this study, the experimental purpose and potential hazards of which were explained in detail to each of them. They were in-patients hospitalized to investigate symptoms which proved to be non-specific. None of the patients were overweight, had a family history of diabetes or presented clinical or laboratory evidence of endocrine and metabolic disorders.

Acute drug administration

One and a half g acetylsalicylic acid (ASA) (Aspirin®, Bayer) was given as a single oral dose to 8 subjects. One hundred mg indomethacin (ID) (Indocid®, Merck Sharp & Dome) was given to another 8 subjects. Eight subjects received a placebo and served as controls. Venous blood samples were obtained from all of the patients prior to and for 3 h following drug administration.

Protracted drug administration

Eight subjects were submitted to an insulin tolerance test (0.1 U crystalline insulin/kg body weight injected iv). Serial venous blood samples were obtained before and for 2 h following insulin injection. The test was repeated in the same subjects after a 4-day treatment with ASA, sustained-release preparation (Cemirit®, Bayer). 3.2 g daily given po in 4 divided doses. The last dose of the drug was administered at 7 a.m. on the day of the post-treatment test. In 8 additional subjects, an insulin tolerance test was performed, as outlined above, before and after a 4-day oral treatment with 300 mg daily of ID. No side effects were noted after acute drug administration. During protracted ASA treatment some patients complained of increased perspiration and sense of stuffiness in the ears. On the fourth day of ID treatment 3 patients complained of headache.
Tests on the patients were invariably started between 9 and 9.30 a.m. after an overnight fast and at least 1 h lying down in bed. A polystyrene cannula was inserted in a forearm vein, kept open by a slow 0.9% saline drip and used as a route for insulin injection and/or for blood sampling. After two basal blood samples had been obtained at 30 min intervals, insulin injection or acute drug administration was performed, according to experimental design. Serial blood samples were obtained thereafter for 2 or 3 h. From each sample an aliquot of blood was taken for glucose determination; the remainder was collected into chilled polystyrene tubes containing EDTA (50 μl of a 2% solution per ml of blood) as anticoagulant, for plasma cortisol determination and EDTA plus aprotinin (Trasylol®, Bayer) (500 units per ml of blood) for plasma ACTH estimation. The tubes were immediately spun in a refrigerated centrifuge and plasma kept deep-frozen until assayed.

Plasma cortisol was determined by a competitive protein-binding radioassay (Baum et al. 1974) using the reagents of a commercial kit (Cortipac®, Radiochemical Centre, Amersham). The sensitivity of the method (least cortisol concentration causing a significant decrease of the binding capacity) is 2.1 μg/100 ml. Reproducibility, expressed as coefficient of variation, was 6.8% and 4.4% (within-assay) and 11% and 7.3% (between-assay) for two samples containing 4.2 μg/100 ml and 39.5 μg/100 ml cortisol, respectively.

Plasma ACTH was measured by radioimmunoassay (Landon & Greenwood 1968) with previous extraction of plasma by porous glass (Ratcliffe & Edwards 1971). Details and validation data of the assay have been reported previously (Cavagnini et al. 1975). Paired plasma samples were always run in the same cortisol or ACTH assay. The possibility that ASA or ID interfered in the cortisol or ACTH determination was excluded by the evaluation of recoveries carried out by the addition of known amounts of cortisol or ACTH to plasma samples of subjects treated with these drugs. Blood glucose was measured by the ferricyanide method using a Technicon Auto Analyzer.

Statistical evaluation of the results was made by Student’s t-test for paired data for the results of the insulin tolerance test and by Scheffe’s test (Miller 1966) for those of acute drug administration.

RESULTS

Acute administration of 100 mg ID or 1.5 g ASA in two groups of 8 subjects each did not cause significant changes in plasma ACTH levels, nor did it modify the circadian downward pattern of plasma cortisol, as compared with that of 8 controls (Fig. 1).

In 8 subjects who were given 300 mg daily ID for 4 days, there was a delay in the plasma ACTH rise in response to insulin hypoglycaemia (51.4 ± 13.91 SE vs. 124.6 ± 15.45 pg/ml at 30 min time, P < 0.05); however, from 45 min thereafter ACTH values were higher than in the basal study, with significant differences at 45 and 60 min time (181.2 ± 14.84 and 140.0 ± 11.08 pg/ml vs. 130.5 ± 16.58 and 93.1 ± 13.23 pg/ml, respectively, P < 0.05) (Fig. 2). As shown in Fig. 2, the cortisol response to hypoglycaemia in the same subjects was moderately reduced after ID treatment, with significant differences at 30 and 45 min (10.7 ± 1.80 and 18.6 ± 1.21 μg/100 ml vs. 16.2 ± 2.01* and 24.0 ± 1.82* μg/100 ml, respectively, * P < 0.05, ** P < 0.01).
ACTH and cortisol patterns after acute oral administration of 100 mg ID (▲—▲), 1.5 g ASA (○——○) and placebo (●——●) in 3 groups of 8 subjects each. Vertical bars indicate SEM.

ACTH and cortisol response to insulin hypoglycaemia before (●——●) and after (○——○) a 4-day administration of 300 mg daily ID to 8 subjects. * P < 0.05, ** P < 0.01.
As shown in Fig. 3, in 8 additional subjects, a 4-day treatment with 3.2 g daily ASA resulted in a significant decrease of the ACTH elevation in response to insulin hypoglycaemia: significant differences were found at 30, 45, 60 and 90 min (45.1 ± 5.50, 94.3 ± 9.06, 85.5 ± 8.90 and 41.7 ± 6.99 pg/ml vs. 110.3 ± 16.73, 121.8 ± 13.23, 118.3 ± 11.18 and 67.0 ± 9.59 pg/ml, respectively, * P < 0.05). In the same subjects, ASA administration did not significantly modify the cortisol response to hypoglycaemia as compared with the pre-treatment values, with the exception of a 15 min delay in onset (12.6 ± 1.86 vs. 17.0 ± 2.9 µg/100 ml at 30 min, * P < 0.05).

Blood glucose levels were slightly increased after ID administration (93.3 ± 6.24 vs. 79.1 ± 5.94 mg/100 ml, N.S.); however, the magnitude of the blood glucose fall induced by insulin was comparable before and after treatment: mean nadir values were 39.9 ± 3.28 vs. 34.2 ± 3.83 mg/100 ml, N.S., respectively. In contrast, after ASA administration, basal blood glucose levels were fairly reduced (74.6 ± 5.71 vs. 82.0 ± 4.72 mg/100 ml, N.S.) but again, their pattern after insulin injection was not significantly different from that in the pre-treatment test: mean nadir values were 31.8 ± 2.8 vs. 35.2 ± 3.7 mg/100 ml, N.S., respectively. As we could judge by the clinical symptoms, the stress induced by hypoglycaemia was similar before and after both ID and ASA administration.

**DISCUSSION**

Interpretation of results of PG studies, chiefly under *in vivo* conditions, is complicated by their intrinsic features such as widespread distribution in the body and diversity of effects depending on the series to which they belong.
the tissue where they act and the duration of their action (Higgins & Braunwald 1972; Honn & Chavin 1976). In our study, acute oral administration of ID or ASA did not influence plasma ACTH and cortisol patterns. We cannot exclude that higher doses of these drugs would have an influence; however, the same dose of ID (100 mg) proved capable of increasing blood glucose and plasma growth hormone levels (Cavagnini et al. 1977). After protracted ID administration, the elevation of plasma ACTH levels in response to hypoglycaemia was greater with respect to pre-treatment but delayed in onset. Bearing in mind that plasma concentration of radioimmunoassayable ACTH may reflect hypophysial secretion, not only of ACTH but also of ACTH-related peptides (β-lipotrophin, endorphin, big ACTH etc.), this result may be interpreted by assuming that ID, due to its complex chemical structure and over 90% binding to plasma proteins (Hvidberg et al. 1972), was unable to cross the blood-brain barrier consistently (Hucker et al. 1966; Dembinska & Gredzinska 1974) and therefore acted chiefly on the pituitary. Here, the PG lack produced by ID could have caused, in a first phase, a block of ACTH release with sustained ACTH synthesis and, therefore, a delay of the ACTH response. When the effect of PG lack is exhausted, increased amounts of ACTH would be secreted. This envisages the possibility that PG in the pituitary promote the release of stored ACTH and inhibit new ACTH synthesis. This view harmonizes with and further defines the concept derived from Hedge's studies (Hedge 1976, 1977; Hedge & Hanson 1972; Hedge & Thompson 1975), that PG, although acting chiefly on the hypothalamus by promoting CRF release, restrain CRF-induced ACTH secretion at the pituitary level. After ID treatment the cortisol response to hypoglycaemia was moderately reduced when compared with the control study. The apparent discrepancy between the ACTH and cortisol pattern may be explained satisfactorily by the concept, supported by direct and indirect experimental evidence (Flack et al. 1969; Flack & Ramwell 1972; Saruta & Kaplan 1972; Gallant & Brownie 1973; Warner & Rubin 1975; Spät & Józan 1975; Shaw & Ramwell 1967; Laychock & Rubin 1975, 1976; Dazord et al. 1974), that PG enhance ACTH-stimulated steroidogenesis in the adrenal cortex. A substantial reduction of plasma ACTH rise together with a slight decrease of cortisol release in response to insulin hypoglycaemia has recently been reported by Beirne & Jubiz (1978) in subjects receiving ID; however, it is difficult to compare the two studies because of marked differences in the experimental design.

After ASA administration for 4 days, plasma ACTH response to hypoglycaemia was diminished with respect to pre-treatment. This may be interpreted by assuming that ASA could effectively exert its anti-PG effect on the hypothalamus, where PG promote CRF and therefore ACTH release. (Hedge & Hanson 1972; Hedge & Thompson 1975; Thompson & Hedge 1976). A primary hypothalamic effect of ASA could be due to its pharmacological
properties (after peripheral administration ASA is rapidly distributed, mainly as salicylic acid, into the cerebral spinal fluid and to a much lesser extent in brain tissue (Woodbury & Fingl 1975; Davison 1971)) or to a high sensitivity of hypothalamic PG-synthetases to this drug (Ferreira & Vane 1974; Patrono et al. 1975). The cortisol response to hypoglycaemia after ASA was unvaried except for a 15 min delay in onset. This finding may be explained by considering that: a) insulin hypoglycaemia does not elicit maximal ACTH secretion but a nearly maximal cortisol secretion b) ASA is far less potent than ID as a PG synthesis inhibitor; thus, ASA might have been capable of blunting CRF-ACTH but not cortisol response c) ACTH secretion after ASA was reduced but may still have induced maximal cortisol release (ACTH response to lysine vasopressin, although definitely lower than that to hypoglycaemia, causes a roughly similar cortisol secretion (Cavagnini et al. 1976)).

The possibility that cortisol displacement from plasma proteins caused by ID and ASA was responsible for our results is unlikely since the results obtained with the two drugs were distinctly different. Instead, the possibility that the effects of ID and ASA were unrelated to PG synthesis inhibition cannot be ruled out.

In conclusion, the results of this study support the concept that PG are involved in the control of the hypothalamic-pituitary-adrenocortical function in man, exerting their effects at each level of this system. In full agreement with most recent views based on animal studies (Hedge 1977), our findings seem to indicate that PG enhance the ACTH and cortisol response to the stress of hypoglycaemia chiefly by increasing hypothalamic CRF release and ACTH-stimulated adrenocortical steroidogenesis, although they may limit hypophyseal ACTH output.

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