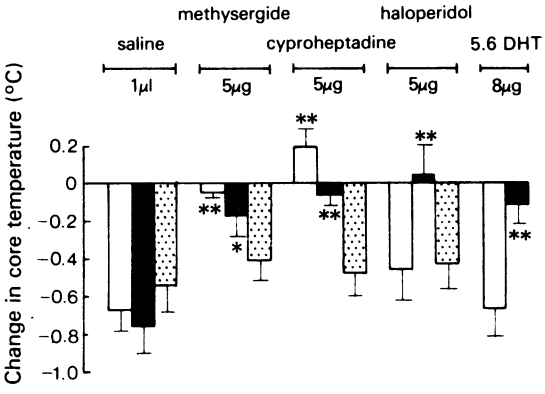

Figure 1 Change in core temperature after intrahypothalamic injection of 5-hydroxytryptamine (20 μg, open column), dopamine (10 μg, closed column) or oxotremorine (1.25 μg, dotted column) in saline or drug pretreated rats. Each column represents the mean maximum change in core temperature ±s.e. mean ($n = 4$ to 18). Significance of difference from appropriate agonist control, $*P < 0.05$, $**P < 0.01$ (Mann-Whitney U test, two tailed).

jected to a heat load were incapable of initiating heat loss in a manner analogous to that following blockade of hypothalamic dopamine receptors (Cox & Lee, 1977). These results support the hypothesis of a dopamine-5-HT link in the central thermoregulatory pathways of the rat.

References

Cox, B. & Lee, T.F. (1977). Do dopamine receptors have a physiological role in thermoregulation? *Br. J. Pharmac.*, **61**, 83–86.

Grabowska, M., Michaluk, J. & Antkiewicz, L. (1973) Possible involvement of brain serotonin in apomorphine induced hypothermia. *Eur. J. Pharmac.*, **23**, 82–89.

Maj, J. & Przewlocka, B. (1975). The action of L-DOPA in rats with the raphe nuclei lesions. *Pol. J. Pharmac. Pharm.*, **27**, Suppl. 151–154.

Przewlocki, R. (1977). The effect of lesions of dopaminergic and serotoninergic systems on apomorphine-induced hypothermia in the rat. *Pol. J. Pharmac. Pharm.*, **29**, 263–270.

Does 5-hydroxytryptamine influence the facilitating effect of fenfluramine on glucose uptake into rat isolated hemidiaphragm?

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Fenfluramine, a clinically useful anti obesity drug, will, in the presence of insulin, increase glucose uptake into rat isolated hemidiaphragm (Bajaj & Vallance-Owen, 1974; Frayn, Hedges & Kirby, 1974) and human gluteus maximus (Kirby & Turner, 1974). Of a series of drugs known to be antagonists of various types of pharmacological receptors, only methysergide appeared to inhibit this effect (Kirby & Turner, 1974), suggesting that 5-hydroxytryptamine may play a role in this phenomenon.

Using the methods described by Frayn *et al.* (1974) on groups of ten rats of the Wistar breed, 5 to 6 weeks old and starved for 24 h prior to sacrifice, we have confirmed that fenfluramine facilitates glucose uptake into rat isolated hemidiaphragm in the presence of insulin 100 μu/ml (mean % glucose uptake increase 77.69, $P < 0.001$).

5-HT (1 μg/ml) in the presence of insulin (100 μu/ml) had no effect on glucose uptake. When 5-HT (1 μg/ml) was added to fenfluramine (100 ng/ml) in the presence of insulin (100 μu/ml), glucose uptake was not significantly different from that with fenfluramine alone. 5-HT (10 μg/ml) also had no effect. However, 5-HT (100 μg/ml) added to fenfluramine (100 ng/ml) in the presence of insulin (100 μu/ml) increased glucose uptake by 26.71% compared to fenfluramine (100 ng/ml) alone ($P < 0.05$). 5-HT (1 μg/ml) added to fenfluramine (50 ng/ml) did not significantly increase glucose uptake compared to fenfluramine (50 ng/ml) alone. 5-HT (100 μg/ml) added to similar fenfluramine concentration in the presence of insulin (100 μu/ml) significantly increased glucose uptake (mean % increase 54.50, $P < 0.025$) when compared to fenfluramine (50 ng/ml) alone in the presence of insulin (100 μu/ml).

The results show that the facilitating effect on glucose uptake seen with fenfluramine in rat isolated hemidiaphragm is significantly increased by 5-HT and are consistent with the hypothesis that the influence of methysergide on the action of fenfluramine on isolated skeletal muscle uptake is mediated by 5-HT receptor blockade.

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References

BAJAJ, S. & VALLENCE-OWEN, J. (1974). Fenfluramine and glucose uptake by muscle. *Horm. Met. Res.*, **6**, 85.

FRAYN, K N., HEDGES, ANNMARIE & KIRBY, M.J. (1974). Stimulation by fenfluramine of glucose uptake into skeletal muscle *in vitro*. *Horm. Met. Res.*, **6**, 86.

KIRBY, M.J. & TURNER, P. (1974). Effect of amphetamine, fenfluramine and norfenfluramine on glucose uptake into human isolated skeletal muscle. *Br. J. Clin. Pharmac.* **1**, 340P–341P.

KIRBY, M.J. & TURNER, P. (1977) The effect of methysergide and other receptor antagonists on fenfluramine induced glucose uptake into the isolated rat hemidiaphragm. *Arch. Int. Pharmacodyn. Ther.*, **225**, 25–28.

Modulation of nuclear oestrogen receptor levels by oestrogen and antioestrogen

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In oestrogen target tissues oestradiol binds to cytoplasmic oestrogen receptors which translocate to the nucleus where they initiate RNA synthesis (Jensen & De Sombre, 1973). During the ensuing protein synthetic phase the cytoplasmic oestrogen receptor pool is replenished (Sarff & Gorski, 1971). Similarly non-steroidal antioestrogens translocate oestrogen receptors to the nucleus, but it has been postulated that antioestrogen-oestrogen receptor complexes inhibit the synthesis of oestrogen receptors therefore making the tissue refractory to oestrogens (Clark, Anderson & Peck, 1973). Some support for this concept has come from studies of antioestrogen action *in vitro* (Horwitz & McGuire, 1978).

If the nuclear levels of antioestrogen-oestrogen receptor complexes represent a labile pool which is being destroyed and then replaced by newly synthesized oestrogen receptors translocated from the cytoplasm, then inhibition of protein synthesis should rapidly reduce nuclear oestrogen receptor levels once this destruction or processing (Horwitz, Koseki & McGuire, 1978) is initiated.

In a preliminary experiment a dose of cycloheximide was determined that would inhibit oestradiol benzoate stimulated uterine cytoplasmic oestrogen receptor synthesis. Cycloheximide (1.25, 2.5, 5 or 10 μg in 0.1 ml saline) was administered s.c. to groups of immature rats (Alderley Park strain, 8 rats/group) every 2 h for 8 h before and 20 h after the s.c. administration of oestradiol benzoate (25 μg in 0.1 ml arachis oil). The 5 μg cycloheximide regimen was found to inhibit oestrogen receptor synthesis without causing severe toxic effects.

The antioestrogens tamoxifen (25 μg, *trans* 1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenyl-but-1-ene) and monohydroxytamoxifen (25 μg 1-(4-β-dimethylaminoethoxyphenyl)-1-(4-hydroxyphenyl)-2-phenyl-but-1-ene) and the oestrogen oestradiol benzoate (25 μg) were administered s.c. to separate groups of immature rats (8 rats/group) every 12 hours. The antioestrogen experiment was repeated with the addition of the 5 μg cycloheximide regimen previously described. Groups of rats were sacrificed 2, 8, 16, 28 and 90 h after the first administration of oestrogen or antioestrogen and uterine nuclear oestrogen receptor concentrations determined using the method of Katzenellenbogen (1975). Nuclear oestrogen receptors increased reaching a maximum 8 h after monohydroxytamoxifen or oestradiol benzoate administration and 16 h after tamoxifen. For monohydroxytamoxifen and oestradiol benzoate treated groups the levels then decreased to control values by 90 h at which time the tamoxifen treated group still had elevated nuclear oestrogen receptor levels. In the presence of cycloheximide, the initial rise in nuclear oestrogen receptors after both antioestrogens was similar to that seen without cycloheximide treatment, however there was a more rapid decrease in nuclear oestrogen receptors in the cycloheximide treated groups, control levels being reached with both compounds.

The results show that antioestrogen-oestrogen receptor complexes are processed in the nucleus in a similar manner to oestrogen receptor complexes. Furthermore the rapid decrease in nuclear antioestrogen-oestrogen receptor complexes observed in the presence of cycloheximide indicates that oestrogen receptors are synthesized after antioestrogenic stimulation and that these receptors upon translocation to the nucleus contribute to the overall pattern of nuclear antioestrogen-oestrogen receptors observed after antioestrogen administration.

References

CLARK, J.H., ANDERSON, J.N. & PECK, E.J. (1973) Oestrogen receptor anti-oestrogen complex: atypical binding by uterine nuclei and effects on uterine growth. *Steroids*, **22**, 707–718.

HORWITZ, K.B., KOSEKI, Y. & MCGUIRE, W.L. (1978). Oestrogen control of progesterone receptor in human breast cancer: role of oestradiol and antioestrogen. *Endocr.*, **103**, 1742–1751.