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USE OF POTENTIATION OF THYROTROPHIN RELEASING HORMONE (TRH)-INDUCED HYPERTHERMIA AS A TEST FOR SCREENING ANTIDEPRESSANTS WHICH ACTIVATE α-ADRENOCEPTOR SYSTEMS

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1 The minimal dose which significantly potentiates the hyperthermia induced by thyrotrophin releasing hormone (TRH, 40 mg/kg i.p.) in mice has been established for tricyclic and other antidepressants (imipramine, amitriptyline, clomipramine, nortriptyline, maprotiline, nomifensine, viloxazine) including a specific inhibitor of noradrenaline (NA) uptake (nisoxetine).

2 The minimal effective dose in this test has been compared with the minimal dose of the same compounds antagonizing reserpine-induced hypothermia. The ratio of the two doses for each substance indicates that potentiation of TRH-induced hyperthermia is, in general, the more sensitive test.

3 A correlation seems to exist between the α-adrenergic effect of antidepressants and the potentiation of TRH-induced hyperthermia. Those antidepressants which do not act on α-adrenergic systems (butriptyline, amineptine, trazodone, danitracen, fluoxetine) are inactive in this test.

4 This property may be used to select antidepressants that activate α-adrenoceptor systems.

Introduction

Although antidepressants are widely used, their mechanism of action has not yet been elucidated. Their therapeutic effect has usually been attributed to inhibition of noradrenaline (NA) or 5-hydroxytryptamine (5-HT) uptake. These effects have been demonstrated by experiments *in vivo* and *in vitro* using antidepressants with tricyclic structures (Glowinski & Axelrod, 1964; Schildkraut, Dodge & Logue, 1969). Results obtained after chronic administration of antidepressants as well as the discovery of new substances with therapeutic activity which do not inhibit monoamine uptake have led to a new hypothesis, according to which antidepressants cause, upon chronic administration, a subsensitivity of the noradrenergic cyclic adenosine 3′,5′-monophosphate (cyclic AMP) generating system (Vetulani, Stawarz, Dingell & Sulser, 1976).

However, although results obtained after chronic administration clearly show a decrease in noradrenergic function, reduced binding to β-adrenoceptors (Wolfe, Harden, Sporn & Molinoff, 1978) and NA stimulated cyclic AMP formation in slices of rat cerebral cortex or limbic forebrain (Vetulani & Sulser, 1975), no behavioural test has to our knowledge demonstrated decreased noradrenergic activity under these conditions. Some authors have shown, on the contrary, increased activity (Maj, Mogilnicka & Klimek, 1979; Maj, Mogilnicka & Kordecka, 1979). Because of these conflicting results obtained after long term administration of antidepressants, studies of the effects of these drugs after acute administration remain of interest.

In a study of the effects of various substances on thyrotrophin (TRH)-induced hyperthermia, we have recently shown that different types of antidepressants (tricyclic, tetracyclic, monoamine oxidase inhibitors and others) potentiate, after acute administration, the hyperthermia induced in mice by intraperitoneal injection of TRH (40 mg/kg) (Desiles, Puech & Rips, 1980). We, therefore, proposed the use of this property in the selection of new antidepressants. This effect is different from the so-called 'analeptic effect' of TRH (Prange, Nemeroff, Loosen, Bissette, Osbahr, Wilson & Lipton, 1979) in which TRH antagonizes the sedation and hypothermia induced by various psychotropic drugs.

In the current study, we have determined the sensitivity of this test by comparing the minimal active dose of antidepressants potentiating TRH-induced hyperthermia with the minimal dose of the same drugs capable of antagonizing reserpine-induced hypothermia, a classical test for selection of antidepressants (Askew, 1963).

TRH-induced hyperthermia seems to depend specifically on the activation of an α-adrenergic system (Desiles, Constans & Rips, 1979; Rips, Desiles

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& Puech, 1979; Desiles & Rips, 1980). We have investigated the effects of new substances which possess antidepressant activity but which seem to have no α-adrenergic activity. This is the case with butriptyline (Pugsley & Lippman, 1974), amineptine (Samamin, Jori, Bernasconi, Morpugo & Garattini, 1977), trazodone (Koe, 1976) and danitracen (Mogilnicka & Klimek, 1979). We have also studied the effects of nisoxetine and fluoxetine which specifically inhibit uptake of NA (Wong, Horn & Bymaster, 1975) and 5-HT (Wong, Horn, Bymaster, Hauser & Molloy, 1974), respectively.

Methods

Female Swiss albino mice weighing 20–24 g were used. Rectal temperature was recorded with a thermistor probe (Bailey Instruments) inserted to a depth of 3 cm. All experiments were performed between 10 h 00 min and 12 h 00 min at an ambient temperature of 21 ± 1°C.

Drugs were dissolved in distilled water or suspended in an aqueous solution of Tween 80 (2%). Imipramine was dispensed from commercially available ampoules and diluted with water as required.

All drugs were administered intraperitoneally, to groups of 12 mice, 30 min before TRH (40 mg/kg i.p.) except for nomifensine which was injected 1 h before the neurohormone. The results indicated for 12 animals are derived from 2 series of experiments. Each experiment was performed on 6 controls, 6 animals treated with TRH and 6 animals treated with TRH and an antidepressant.

Only temperatures measured 15 min after administration of TRH (or vehicle) are shown in the tables. Temperatures of animals pretreated with the compounds under study before receiving TRH were compared with those of animals injected with vehicle only before TRH. Temperatures of animals receiving the experimental substance followed by vehicle or TRH following vehicle were compared with those of controls receiving two injections of vehicle only.

The means were compared with Student's t test. The statistical calculations concerned groups treated under strictly identical experimental conditions, the same day at the same hour. Although the hyperthermia induced by TRH varied between experiments, the strict experimental conditions permit statistical analysis because of the slight variance among values obtained with TRH within each experiment.

The minimal effective dose (MED) was the smallest dose which significantly ($P<0.05$) increased TRH-induced hyperthermia.

For the reserpine antagonism test, drugs were administered intraperitoneally, to groups of 6 mice, 17 h after injection of reserpine (1.5 mg/kg i.p.). Reserpine was dissolved in an aqueous solution of ascorbic acid (5%). Temperatures were measured 30 min, 1 h and 2 h after administration of the drugs.

The MED dose was the smallest dose having a significant effect ($P<0.05$) compared with reserpine-treated controls.

The following drugs were used: amineptine (Servier); amitriptyline (Roche); butriptyline (Auclair); clomipramine (Ciba); danitracen (Dr K. Thomae. GmbH.); fluoxetine (Lilly); imipramine (Geigy); maprotiline (Ciba); nisoxetine (Lilly); nomifensine (Hoechst); nortriptyline (Lilly); reserpine (Aldrich); TRH (synthesized by INSERM U 98); trazodone (Instituto di Ricerca F. Angelini); viloxazine, HCl (ICI).

Results

Minimal effective doses of compounds potentiating TRH-induced hyperthermia: comparison with anti-reserpine activity

The dose-dependent effects of various antidepressants, tricyclic (imipramine, amitriptyline, nortriptyline, clomipramine), tetracyclic (maprotiline) or other drugs (nomifensine, viloxazine), on TRH-induced hyperthermia are presented in Table 1.

It was previously verified (Desiles *et al.*, 1980) that these substances do not affect temperature by themselves. Nisoxetine, which was not studied in our previous paper, also potentiates TRH hyperthermia in a dose-dependent manner (Table 2).

The MED of these compounds are listed in Table 3.

When these doses are compared with those obtained with the reserpine test under our experimental conditions, it is clear that, in all cases, the former test is more sensitive than the latter. The ratio of active doses in the two tests ranges between 2.4 for nortriptyline and 64 for nisoxetine. The order of activity of the drugs differs in the two tests.

Effect on TRH-induced hyperthermia of antidepressants that do not affect α-noradrenergic systems

The results obtained are presented in Table 4. None of the compounds studied significantly potentiate TRH hyperthermia. A decrease in the effect of TRH is even observed in the case of trazodone which, when administered alone, provokes a hypothermia.

Amineptine, which itself increases temperature significantly, does not potentiate TRH-induced hyperthermia. The effect of butriptyline at a dose of 20 mg/kg is at the limit of significance ($t = 1.88$) because the variance among TRH values is slightly

Table 1 Effects of antidepressant drugs on thyrotrophin releasing hormone (TRH, 40 mg/kg i.p.)-induced hyperthermia

	Dose (mg/kg i.p.)	*Controls*	*TRH*[¶]	*TRH + drug*[†]
Imipramine	0.25	37.5 + 0.1	39.0 ± 0.1***	39.3 ± 0.1[NS]
	0.50			39.6 ± 0.2**
	1.0			39.6 ± 0.2*
Amitriptyline	0.1	37.8 ± 0.2	38.5 ± 0.2*	38.5 ± 0.2[NS]
	0.2			39.2 ± 0.2*
	0.4			39.1 ± 0.2*
	0.8			39.4 ± 0.2**
Clomipramine	0.375	37.5 ± 0.1	38.3 ± 0.2***	38.5 ± 0.2[NS]
	0.75			38.9 ± 0.1*
	1.5			38.9 ± 0.1*
Nortriptyline	0.06	37.3 ± 0.1	38.6 ± 0.1***	38.8 ± 0.2[NS]
	0.125			39.3 ± 0.1***
	0.25			39.6 ± 0.1***
Maprotiline	1.0	37.8 ± 0.2	39.3 ± 0.1***	39.4 ± 0.3[NS]
	2.0			40.0 ± 0.1**
	4.0			40.0 ± 0.1**
Nomifensine	0.375	37.6 ± 0.1	38.3 ± 0.1***	38.6 ± 0.2[NS]
	0.75			39.4 ± 0.1***
	1.5			39.3 ± 0.2***
Viloxazine	0.5	37.8 ± 0.2	38.3 ± 0.2*	38.6 ± 0.2[NS]
	1.0			39.6 ± 0.1***
	2.0			39.6 ± 0.2***

Groups were composed of 12 animals. Temperatures (means ± s.e.means) were measured 15 min after TRH administration.
[¶] Comparison with control; [†] comparison with TRH.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$; [NS] = not significant.

higher in this series of experiments. However, since the effect is observed at a higher dose than for the antidepressants listed in Table 1, we have classified this substance among those antidepressants inactive in the test.

Discussion

In a preliminary study (Desiles *et al.*, 1980), we showed that the majority of antidepressants, whether tricyclic or not, potentiate TRH-induced hyperthermia. The present work performed on a greater number of compounds confirms the interest of this test which is sensitive to very low doses of antidepressants. The minimal effective doses are much lower than those obtained with the reserpine test. They are also lower than the doses active in the new Porsolt test identifying antidepressants (Porsolt, Anton, Blavet & Jalfre, 1978). For example, imipramine, amitriptyline and viloxazine are active at doses of 30, 15 and 30 mg/kg, respectively, in the Porsolt test (Porsolt, Bertin & Jalfre, 1977) while minimal effective doses in the TRH-induced hyperthermia test are 0.5, 0.2 and 1 mg/kg respectively. Benzatropine and phenobarbitone which are active in the Porsolt test (Browne, 1979; Schechter & Chance, 1979) do not reinforce TRH-induced hyperthermia (results not shown).

The potentiation of TRH-induced hyperthermia by antidepressants seems related to the effect of these substances on an α-adrenergic system. Although several antidepressants have profound anticholinergic effects which might affect the results obtained, the

Table 2 Effect of nisoxetine on thyrotrophin releasing hormone (TRH, 40 mg/kg i.p.)-induced hyperthermia

	Dose (mg/kg i.p.)	*Controls*	*Drug*[¶]	*TRH*[¶]	*TRH + drug*[†]
Nisoxetine	0.16	37.2 ± 0.2	37.5 ± 0.2	38.6 ± 0.2***	39.0 ± 0.3[NS]
	0.31		37.7 ± 0.2*		39.3 ± 0.2*

For details see footnote to Table 1.

Table 3 Comparison between minimal effective doses (MED) potentiating thyrotrophin releasing hormones (TRH)-induced hyperthermia and antagonizing reserpine-induced hypothermia: ratio of effective doses

	MED TRH test (mg/kg)	*MED Reserpine test* (mg/kg)	*MED Reserpine test* / *MED TRH test*
Imipramine	0.5	5.0	10.0
Amitriptyline	0.2	1.25	6.25
Clomipramine	0.75	*	—
Nortriptyline	0.125	0.31	2.4
Maprotiline	2.0	20.0	10.0
Nomifensine	0.75	2.5	3.33
Viloxazine	1.0	2.5	2.5
Nisoxetine	0.31	20.0	64.0

* Clomipramine (1.25 to 20 mg/kg) had no significant effect.

fact that atropine and scopolamine do not affect TRH hyperthermia (unpublished results) suggests that these properties do not seem to be implicated in the results obtained. Those new substances which have been described as having antidepressant properties and which do not have effects of this type (see introduction) do not potentiate TRH hyperthermia. On the other hand, nisoxetine which is a specific inhibitor of NA uptake (Wong *et al.*, 1975) is active at very low doses in this test. There is a difficulty of interpretation when the antidepressant, like trazodone and danitracen, produces a marked fall in body temperature when given alone. The criterion chosen for potentiation of TRH-induced hyperthermia is that body temperature after 'antidepressant + TRH' should be significantly greater than after 'saline + TRH'. We were not concerned with the 'analeptic effect' of TRH which antagonizes both sedation and hypothermia induced by various barbiturates, chloral hydrate, reserpine, chlorpromazine and diazepam (Prange *et al.*, 1979). In this case, the greater the hypothermia induced by the substance administered with TRH, the greater is the increase in temperature observed. We justify our criterion by the fact that no correlation seems to exist between the proper effect of a substance on the body temperature and modification of TRH-induced hyperthermia. Thus amphetamine, L-DOPA and *p*-chloroamphetamine, at doses causing highly significant hypothermia, potentiate TRH-induced hyperthermia. Apomorphine produced at 1 mg/kg a highly significant hypothermia of 4.2°C but does not affect

Table 4 Effects on thyrotrophin releasing hormone (TRH)-induced hyperthermia of drugs that do not affect α-adrenergic systems

	Dose (mg/kg i.p.)	*Controls*	*Drug*[¶]	*TRH*[¶]	*TRH + drug*[†]
Butriptyline	0	38.1 ± 0.1		38.8 ± 0.3**	
	5		38.1 ± 0.1		39.0 ± 0.2^{NS}
	10		38.0 ± 0.2		39.0 ± 0.2^{NS}
	20		37.8 ± 0.1		39.4 ± 0.2^{NS}
Amineptine	0	37.7 ± 0.1		38.9 ± 0.2***	
	10		38.5 ± 0.1***		38.9 ± 0.2^{NS}
	20		38.3 ± 0.1**		38.9 ± 0.1^{NS}
	40		37.3 ± 0.3		39.2 ± 0.1^{NS}
Trazodone	0	38.0 ± 0.1		39.5 ± 0.2***	
	5		37.1 ± 0.2**		38.4 ± 0.2**
	10		36.6 ± 0.2***		37.9 ± 0.2***
	20		35.1 ± 0.3***		36.8 ± 0.4***
Danitracen	0	37.9 ± 0.1		39.0 ± 0.2***	
	5		34.8 ± 0.2***		38.6 ± 0.3^{NS}
	10		35.1 ± 0.3***		38.5 ± 0.6^{NS}
	20		34.3 ± 0.3***		38.0 ± 0.6^{NS}
Fluoxetine	0	38.1 ± 0.1		39.2 ± 0.2***	
	5		38.0 ± 0.2		39.2 ± 0.2^{NS}
	10		38.0 ± 0.2		39.3 ± 0.2^{NS}
	20		36.6 ± 0.4**		39.3 ± 0.1^{NS}

For details see footnote to Table 1.

TRH-induced hyperthermia (Rips *et al.*, 1979). Amineptine which, on the contrary, induces hyperthermia, does not potentiate TRH-induced hyperthermia. Some antidepressants, imipramine, nortriptyline, nomifensine and viloxazine, at high doses which cause significant hypothermia, also potentiate TRH-induced hyperthermia (results not shown). These arguments demonstrate that the hypothermia inducing properties of a substance do not necessarily prevent its potentiation of TRH-induced hyperthermia.

In conclusion the potentiation of TRH-induced hyperthermia, a property which is shared only by substances affecting adrenergic systems, may be used as a rapid method for detecting α-adrenergic effects of substances whose mechanism of action is unknown.

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