ELSEVIER

European Journal of Pharmacology 433 (2001) 73–79

ejp

www.elsevier.com/locate/ejphar

Testosterone mediates sex difference in hypothermia and cholinesterase inhibition by rivastigmine

Rui-Hua Wang, Donna Schorer-Apelbaum, Marta Weinstock*

Department of Pharmacology, School of Pharmacy, Hebrew University, Hadassah Medical Centre, Ein Kerem, Jerusalem 91120, Israel

Received 29 August 2001; received in revised form 25 October 2001; accepted 30 October 2001

Abstract

The fall in body temperature and inhibition of hypothalamic cholinesterase induced by rivastigmine (a pseudo-reversible carbamate inhibitor) were compared in male and female rats. In males, 1.5 mg/kg lowered body temperature by 1 °C and in females by 3.2 °C ($P < 0.001$) and inhibited cholinesterase by 65% and 74%, respectively ($P < 0.05$). Pilocarpine (20 mg/kg) decreased body temperature by 1.1 °C in males and 1.9 °C in females ($P < 0.05$). Orchidectomy, but not ovariectomy, abolished the sex difference in the hypothermic effect of pilocarpine and the enzyme inhibition induced by rivastigmine, but not in its effect on body temperature. Testosterone (10 mg/rat) decreased the cholinesterase inhibition and the temperature reduction induced by rivastigmine in gonadectomised males and females, but that induced by pilocarpine in males only. In conclusion, rivastigmine causes less inhibition of cholinesterase because testosterone may interfere with its entry into the brain. Testosterone may further decrease the temperature-lowering effect of rivastigmine and acetylcholine receptor agonists in males by an action at a receptor level.

Keywords: Body temperature; Cholinesterase inhibition; Gonadectomy; Pilocarpine; Rivastigmine; Testosterone

1. Introduction

Several independent studies of rodents have shown that organophosphorus and carbamate cholinesterase inhibitors have a different effect in males and females, but that the direction is inconsistent. In particular, the sex difference in the hypothermic effect varies with the particular agent. Thus, chlorpyrifos, an organophosphorus insecticide, produces a greater fall in body temperature in female than in male rats (Gordon et al., 1997), while di-isopropyl-fluorophosphonate (DFP) is more effective in males (Overstreet et al., 1979). In the latter study, pilocarpine, a muscarinic receptor agonist, caused a greater degree of hypothermia in females than in males.

The effect of these drugs on body temperature results from stimulation of muscarinic receptors in the pre-optic area of the hypothalamus, promoting increased heat loss through peripheral vasodilatation (Gordon, 1994). This can be prevented by prior treatment with scopolamine or atropine (Sen and Bhattacharya, 1991). Although it is presumed that cholinesterase inhibitors lower body temperature via increased levels of acetylcholine, wide discrepancies have been reported between the magnitude of hypothermia induced by physostigmine and paraoxon and their respective inhibition of cholinesterase in the hypothalamus (Coudray-Lucas et al., 1981; Gordon, 1994).

To our knowledge, there has been only one report of a sex difference in the action of cholinesterase inhibitors in human subjects. Physostigmine increased plasma cortisol and adrenocorticotropin more in normal elderly women and those with Alzheimer's disease than in men, although plasma levels of the drug did not differ (Peskind et al., 1996). We have recently found that physostigmine causes greater inhibition of cholinesterase in the brain, but not in the heart and plasma, of female than of male rats (Wang et al., 2000). We also showed that rivastigmine, another carbamate cholinesterase inhibitor currently used for the treatment of Alzheimer's disease (Rosler et al., 1999), was more effective in antagonising spatial memory deficits in female than in male rats. This effect was associated with a greater inhibition of cholinesterase in the cortex and hippocampus of females (Wang et al., 2000). Rivastigmine is more suitable than chlorpyrifos for studying the relationship between enzyme inhibition and hypothermia because it is inactivated entirely by its target enzymes, acetyl and butyrylcholinesterase

* Corresponding author. Tel.: +972-2-6758731; fax: +972-2-6758741.
E-mail address: martar@cc.huji.ac.il (M. Weinstock).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01498-4

(Farlow and Hake, 1998), and not by cytochrome *P*450 or liver carboxylesterase, which may be sex hormone-dependent (Ma and Chambers, 1994; Moser et al., 1998). Moreover, in contrast to physostigmine, rivastigmine is not readily detached from the enzymes during the extraction procedures preceding the measurement of cholinesterase activity ex vivo. Thus, one may expect to obtain a more accurate estimate of enzyme inhibition by doses that cause a significant fall in body temperature.

The aims of the present study were: (a) to examine the relationship between hypothalamic cholinesterase inhibition and the fall in body temperature induced by rivastigmine in male and female rats; (b) to investigate the role of sex steroids in the mediation of the sex difference by examining the influence of castration and hormone replacement on these pharmacological effects of rivastigmine.

2. Materials and methods

2.1. Animals

The study was performed according to the guidelines of the University Committee for Institutional Animal Care, based on those of the National Institutes of Health, USA. Male and female Sprague–Dawley rats, aged 9–11 weeks, weighing 220–300 g, were purchased from Harlan, Jerusalem. The rats were housed four per cage for 1 week in the Animal House before the experiment. The ambient temperature was 23 ± 1 °C and there was a 12-h diurnal light cycle (lights on at 0600 h and off at 1800 h). Body temperature was measured in a room adjacent to that in which the rats were housed and had the same ambient conditions.

2.2. Experimental procedures

2.2.1. Measurement of effects of rivastigmine and pilocarpine on body temperature

A digital thermistor probe was covered with a thin layer of lignocaine gel to reduce discomfort and inserted into the rectum to a depth of 6 cm. Rivastigmine, (0.75, 1.5, 2.5 and 3.5 mg/kg) or pilocarpine (20 mg/kg) was injected s.c in groups of five to six rats per dose and sex. Temperature was measured before and at 15-min (pilocarpine) or 30-min (rivastigmine) intervals after injection up to 3 h.

2.2.2. Determination of optimal conditions for obtaining effect of gonadectomy and testosterone administration on hypothermic effect of rivastimine

Orchidectomy was performed under ether anaesthesia in five groups of six rats. One group of intact rats served as a control. Three groups and the controls were injected with vehicle and the effect of rivastigmine (1.5 mg/kg) on body temperature was assessed 1, 2 and 3 weeks, respectively, after orchidectomy. In the remaining two groups, testosterone (5 or 10 mg/rat) was injected i.m. 2 weeks after orchidectomy, and the effect of rivastigmine on body temperature was measured 1 week later. Both doses of testosterone resulted in serum hormone levels 1 week after injection that were a little higher than those in intact rats.

2.2.3. Effect of gonadectomy and testosterone administration on the effect of rivastigmine and pilocarpine on body temperature

Groups of male and female rats were castrated under ether anaesthesia and further groups of sham-operated males and females served as controls. The hypothermic effect of rivastigmine (1.5 mg/kg) (10–12 rats per group) and pilocarpine (20 mg/kg) (5–6 rats per group) was measured in rats of each sex and that of rivastigmine (0.75 mg/kg) was assessed in females only (5–6 rats per group), 3 weeks after castration and injection of vehicle or testosterone (10 mg/rat) as described above.

2.3. Measurement of cholinesterase inhibition in the hypothalamus

Saline (1 ml/kg) or rivastigmine (1.5 mg/kg) was injected s.c. into male and female rats (five to nine per group) and additional groups of females were given 0.75 mg/kg, since this lowered body temperature to the same extent as 1.5 mg/kg in males. The rats were decapitated after 60 min and the brains were rapidly removed. The hypothalamus was dissected out, weighed and homogenised in 0.1 M phosphate buffer (33 mg/ml), pH 8.0, containing 1% Triton. Trunk blood was collected and separated into plasma. Total enzyme activity, almost entirely butyrylcholinesterase in plasma (Cullumbine, 1963) and more than 90% acetylcholinesterase in the brain (Hobbiger and Lancaster, 1971), was measured in 25-μl aliquots of enzyme homogenates in a total volume of 1 ml by the method of Ellman et al. (1961) using acetylthiocholine as a substrate. The percent inhibition of the enzyme by rivastigmine was calculated by comparison with cholinesterase activity in the hypothalamus and plasma taken from rats injected with saline under the same conditions and at the same time of day to avoid diurnal variations in cholinesterase activity (Moudgil and Kanungo, 1973).

2.3.1. Effect of gonadectomy and hormone replacement on cholinesterase activity and its inhibition by rivastigmine

Male and female rats were castrated and given injections of vehicle or testosterone as described in Section 2.2.3. Cholinesterase activity was measured in the hypothalamus and plasma after injection of saline or rivastigmine (1.5 mg/kg) in rats of each sex, or 0.75 mg/kg in females only, as described in Section 2.3.

2.4. Drugs

Pilocarpine nitrate was from Sigma (MO, USA) and rivastigmine bitartrate (ENA713) from Novartis (Basle, Switzerland). Testosterone enanthate was from Schering

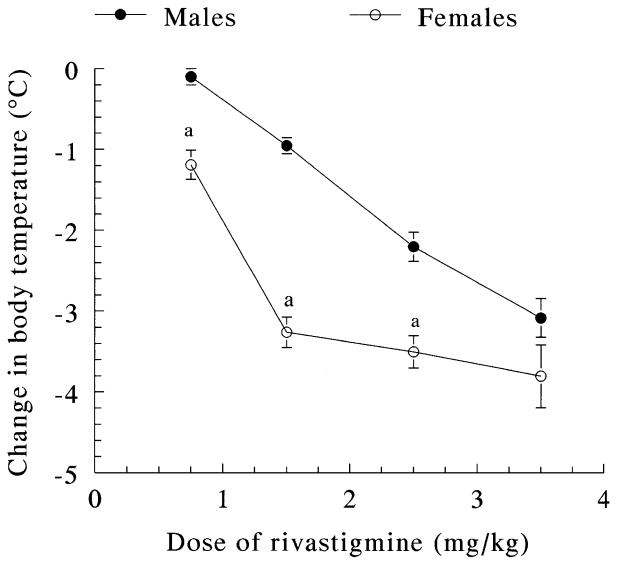

Fig. 1. Dose–response relationship for the fall in body temperature induced by rivastigmine in male and female rats. Significantly different from males, $^{a}P<0.01$.

(Berlin, Germany). All doses are expressed in terms of mg/kg body weight of the salt. Testosterone was dissolved in alcohol, one part, and diluted with arachis oil, nine parts. Control rats received vehicle injections consisting of alcohol and arachis oil in the same proportions.

2.5. Data analysis

The fall in body temperature induced by rivastigmine and pilocarpine was compared by analysis of variance (ANOVA) (intact, gonadectomised and gonadectomised plus testosterone). Differences in percent inhibition by rivastigmine of cholinesterase in the hypothalamus and plasma were analysed by ANOVA for sex, pretreatment and tissue. When significant differences were found, Duncan's test for multiple comparisons of individual groups was performed. The level of statistical significance was $P<0.05$. All data are expressed as the mean values for the group ± S.E.M.

3. Results

3.1. Hypothermic effect of rivastigmine in male and female rats

The resting body temperature of male and female rats did not differ significantly and was 38.1 ± 0.1 and 37.9 ± 0.2 °C, respectively. The hypothermic effect of different doses of rivastigmine in males and females is shown in Fig. 1, and the time course of the fall in body temperature induced by rivastigmine (1.5 mg/kg) and pilocarpine (20 mg/kg) is shown in Fig. 2. The fall in body temperature was much greater in females than in males at all doses ranging from 0.75 to 2.5 mg/kg, respectively. The maximum reduction in body temperature occurred 60–90 min after injection in rats of both sexes. The extent of the hypothermic effect of pilocarpine (20 mg/kg) did not differ from that of rivastigmine (1.5 mg/kg) in males, but was significantly smaller in females. Thus, although statistically significant, the sex difference in the hypothermic effect of pilocarpine was clearly less than that of rivastigmine.

3.1.1. Effect of gonadectomy and hormone replacement on the hypothermic effect of rivastigmine and pilocarpine

Neither orchidectomy nor ovariectomy significantly changed the basal body temperature of rats maintained at an ambient temperature of 23 °C. The values for gonadectomized males and females 3 weeks after surgery were 37.8 ± 0.1 and 37.6 ± 0.3 °C, respectively. The body temperature of castrated males and females was unaffected by

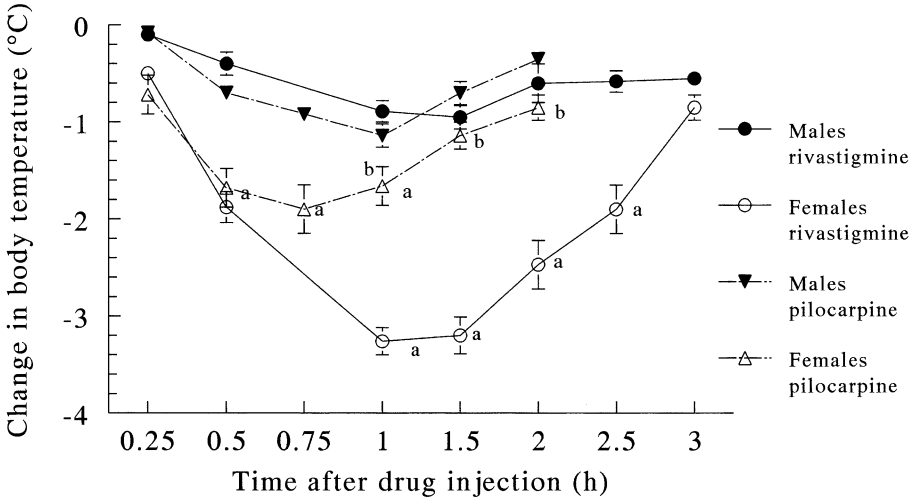

Fig. 2. Time course of hypothermia induced in male and female rats by rivastigmine 1.5 mg/kg and pilocarpine 20 mg/kg. Significantly different from respective males, $^{a}P<0.05$; significantly different from females treated with rivastigmine $^{b}P<0.05$.

testosterone. A significant increase in the hypothermic effect of rivastigmine was seen in males 2 and 3 weeks, but not 1 week after castration. The hypotherma induced by rivastigmine in castrated rats was restored to control levels by testosterone (5 or 10 mg/rat) 1 week after injection (Fig. 3).

Two-way ANOVA for differences produced by gonadectomy and testosterone injection in the peak hypothermic effect of rivastigmine (1.5 mg/kg) revealed a significant effect of sex [$F(1,60)=77.9$, $P<0.0001$], pretreatment [$F(2,60)=6.76$, $P<0.005$] and a sex × pretreatment interaction [$F(2,60)=3.67$, $P<0.05$]. Post hoc tests showed a marked difference in the effect of the drug in intact males and females. It also showed that gonadectomy significantly increased the peak fall in temperature induced by rivastigmine in males but not in females (Fig. 4). The fall in temperature remained significantly different between gonadectomised males and females. Although testosterone reduced the hypothermic effect of rivastigmine in gonadectomised rats of either sex, the sex difference was still pre-sent. Ovariectomy still had no effect when a smaller dose of rivastigmine (0.75 mg/kg) was given to females to produce the same fall in temperature to that seen in males given 1.5 mg/kg. However, testosterone decreased the hypothermic effect of this dose in ovariectomised females to the same level as in orchidectomised males (Fig. 4).

Two-way ANOVA on the hypothermic effect of pilocarpine (20 mg/kg) in the different treatment groups revealed a significant effect of sex [$F(1,30)=12.8$, $P<0.001$], no effect of pretreatment [$F(2,30)=2.0$, $P=0.16$] and a significant sex × pretreatment interaction [$F(2,30)=8.5$, $P<0.0025$]. In contrast to rivastigmine, the sex difference was completely abolished by orchidectomy, so that the fall in body temperature in males no longer differed from that in intact or ovariectomised females (Fig. 5). Furthermore, testosterone restored the effect of pilocarpine in orchidectomised males to that in intact animals, but did not change that in ovariectomised females.

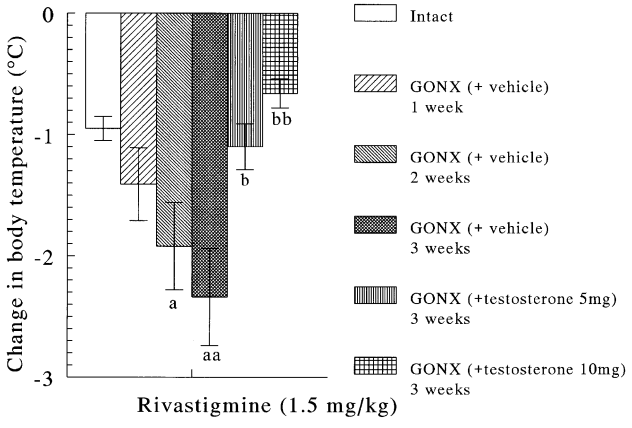

Fig. 3. Influence of time interval after castration and testosterone replacement on the fall in body temperature induced by rivastigmine, 1.5 mg/kg, in male rats. GONX = gonadectomy. Significantly different from intact males, $^{a}P<0.05$; $^{aa}P<0.01$; significantly different from gonadectomised males + vehicle, $^{b}P<0.05$; $^{bb}P<0.01$.

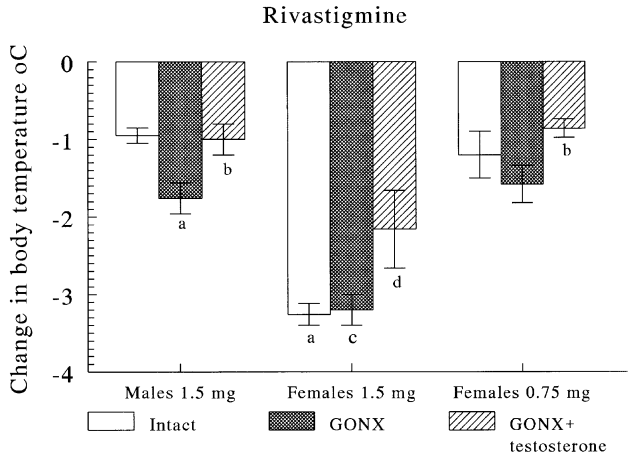

Fig. 4. Effect of gonadectomy and testosterone replacement on the fall in body temperature induced by rivastigmine in male and female rats. Significantly different from intact males, $^{a}P<0.01$; significantly different from gonadectomised males and females, $^{b}P<0.05$; significantly different from gonadectomised males + testosterone, $^{c}P<0.05$.

3.2. Cholinesterase inhibition by rivastigmine in the hypothalamus

ANOVA on resting cholinesterase activity in the hypothalamus did not reveal any significant differences between intact males and females, nor was it affected by gonadectomy or testosterone [$F(5,29)=0.22$, $P=0.95$]. As reported by Overstreet et al. (1979), plasma cholinesterase activity in intact females was about double that in males and signifi-

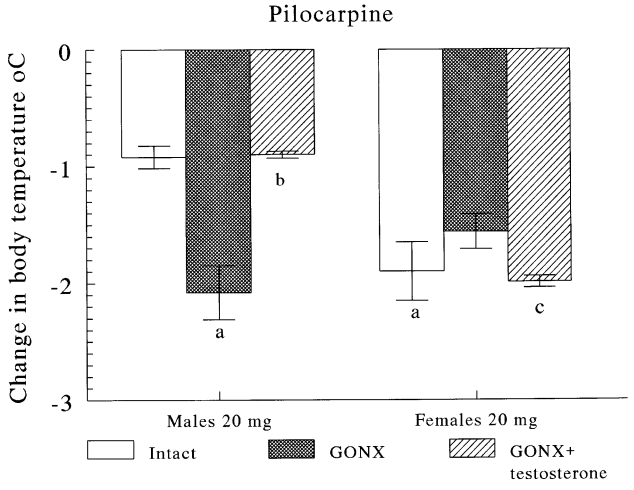

Fig. 5. Effect of gonadectomy and testosterone replacement on the fall in body temperature induced by pilocarpine, 20 mg/kg, in male and female rats. Significantly different from intact males, $^{a}P<0.01$; significantly different from intact females, $^{b}P<0.05$; significantly different from gonadectomised males + testosterone, $^{c}P<0.05$.

Table 1
Cholinesterase activity[a] in the hypothalamus and plasma of intact male and female rats and after gonadectomy and hormone replacement

Tissue	Intact		GONX[b]		GONX + testosterone[c]	
	Male	Female	Male	Female	Male	Female
Hypothalamus	27.8 ± 1.6	27.4 ± 1.2	25.8 ± 2.7	27.8 ± 1.0	27.8 ± 1.4	28.0 ± 0.3
Plasma	1.11 ± 0.1	2.27 ± 0.24[d]	1.14 ± 0.09	1.66 ± 0.14[e]	1.00 ± 0.04	1.25 ± 0.16

[a] Activity of cholinesterase in μmol of acetylthiocholine hydrolysed/g/min.
[b] Three weeks after gonadectomy (GONX).
[c] One week after injection of testosterone. Significantly different from intact males.
[d] $P < 0.01$; significantly different from males.
[e] $P < 0.05$; significantly different from intact females.

cantly reduced by ovariectomy. ANOVA on plasma cholinesterase activity in six groups of rats was [$F(5,30) = 8.59$, $P < 0.0001$]. Post hoc tests revealed that this was due to the difference between intact males and females and between intact and ovariectomised females. Neither orchidectomy nor testosterone administration had a significant effect on plasma cholinesterase activity (Table 1).

Two-way ANOVA for cholinesterase inhibition by rivastigmine (1.5 mg/kg) in the hypothalamus of intact, gonadectomised males and females, with and without testosterone revealed a significant effect of sex [$F(1,30) = 7.90$, $P < 0.01$], pretreatment [$F(2,30) = 12.95$, $P < 0.0001$] but no sex × treatment interaction [$F(2,30) = 2.6$ $P = 0.1$]. Rivastigmine (1.5 mg/kg) caused significantly greater cholinesterase inhibition in the hypothalamus of intact females than of males. Gonadectomy significantly increased cholinesterase inhibition in males but not in females ($P < 0.05$) and abolished the sex difference. Testosterone restored the cholinesterase inhibitory activity of rivastigmine in gonadectomised males to that in intact males and also reduced the activity in females to below the level in intact and ovariectomised animals.

ANOVA for percent inhibition of plasma cholinesterase by rivastigmine (1.5 mg/kg) in the three groups of male and females rats did not detect any significant differences between the groups [$F(5,30) = 1.01$, $P = 0.43$]. However, when compared to the percent inhibition of cholinesterase in the hypothalamus of the same rats, ANOVA for the factors tissue, sex and pretreatment revealed a highly significant effect of tissue [$F(1,64) = 57.8$, $P < 0.0001$] and a significant area × pretreatment interaction [$F(1,57) = 3.98$, $P < 0.025$]. While percentage inhibition of cholinesterase in the hypothalamus was significantly greater than that in plasma in intact males and females and after gonadectomy, it did not differ after testosterone administration (Table 2). A similar tissue × pretreatment interaction was seen when cholinesterase inhibition was analysed in the hypothalamus and plasma of females given rivastigmine (0.75 mg/kg), [$F(1,37) = 5.7$, $P < 0.01$]. This was due to a decrease in cholinesterase inhibition in the hypothalamus and an increase in plasma as a result of testosterone administration to ovariectomised rats.

4. Discussion

The hypothermia induced by cholinesterase inhibitors and cholinergic agonists is believed to result from activation of muscarinic receptors in the anterior hypothalamus (Gordon, 1994). However, previous studies with a carbamate physostigmine, and organophosphorus cholinesterase inhibitors, DFP, soman and paraoxon showed considerable differences in the extent of enzyme inhibition in the brain for the same decrease in body temperature (Gordon, 1994). Sex differences have also been reported for brain cholinesterase inhibition by chlorpyrifos and DFP and for the hypothermic

Table 2
Effect of gonadectomy and testosterone on cholinesterase (%) inhibition by rivastigmine in the hypothalamus and plasma of male and female rats

Dose (mg/kg)	Intact		GONX		GONX + testosterone	
	Male	Female	Male	Female	Male	Female
1.5						
Hypothalamus	65.1 ± 0.9	73.9 ± 1.5[a]	71.3 ± 1.6[b]	72.1 ± 1.2	61.4 ± 3.0[c]	64.4 ± 1.1[c]
Plasma	51.2 ± 2.4[d]	47.7 ± 7.8[d]	49.8 ± 4.4[d]	56.6 ± 3.7[d]	50.7 ± 3.1	59.4 ± 4.2
0.75						
Hypothalamus	–	64.5 ± 1.8	–	62.1 ± 3.0	–	48.1 ± 3.9[c]
Plasma	–	48.7 ± 2.4[d]	–	50.8 ± 7.1	–	57.9 ± 1.0

NT = not tested.
[a] Significantly different from males, $P < 0.05$.
[b] Significantly different from intact rats, $P < 0.05$.
[c] Significantly different from GONX.
[d] Significantly different from respective value for hypothalamus, $P < 0.05$.

effects of these drugs (Overstreet et al., 1979; Gordon et al., 1997). These sex differences were investigated in more detail in the present study, in which we also attempted to relate the fall in body temperature to cholinesterase inhibition in the hypothalamus, rather than in the whole brain (Overstreet et al., 1979; Gordon, 1994) or serum (Gordon and Fogelson, 1993). We used the carbamate, rivastigmine, which produces a more stable interaction than physostigmine with the enzyme and therefore gives a more accurate estimate of inhibition after removal of the tissue prior to measurement. Moreover, rivastigmine is not metabolised by liver enzymes, as are chlopyrifos and DFP, which could explain the possible sex difference in their actions (Ma and Chambers, 1994; Moser et al., 1998).

The smallest dose of rivastigmine that caused a significant fall in body temperature (1 °C) was 1.5 mg/kg in males and 0.75 mg/kg in females. These doses inhibited cholinesterase in the hypothalamus by 65%. The findings with a carbamate support the observation of Gordon (1994) for organophosphorus compounds, namely that cholinesterase must be inhibited by more than 60% before any significant effect on body temperature is seen. The low (15–20%) inhibition of the brain enzyme by physostigmine (Gordon, 1994) may have been due to its removal from the enzyme during extraction from tissues prior to measurement.

In the present study, rivastigmine produced a much greater fall in body temperature in female than in male rats at doses ranging from 0.75 to 2.5 mg/kg. A dose of 1.5 mg/kg in females reduced body temperature by 3.2 °C and inhibited hypothalamic cholinesterase by 74%. This sex difference in enzyme inhibition is not due to a greater affinity of the drug for cholinesterase in the female brain since it was not seen when rivastigmine was added to enzyme preparations from the brains of male and female rats (Wang et al., 2000). The greater effect in females resembles that reported by Gordon et al. (1997) for chlorpyrifos and may result from a similar mechanism. However, while a difference in metabolism by liver enzymes could contribute to the smaller pharmacological effect of chlorpyrifos in males (Ma and Chambers, 1994; Moser et al., 1998), this does not explain the sex difference seen with rivastigmine. Plasma cholinesterase inhibition did not differ in males and females, indicating that the blood levels were similar.

As reported for chlorpyrifos (Gordon et al., 1997), orchidectomy also increased the hypothermic effect of rivastigmine. This differed from the effect of DFP on body temperature, which was greater in males and was increased in females by ovariectomy (Overstreet et al., 1979, 1981). Removal of estrogen increased the concentration of DFP in the brain and caused greater cholinesterase inhibition. At the same time, butyrylcholinesterase activity in plasma decreased. The authors suggested that the higher levels of butyrylcholinesterase in females serve to trap the drug, thereby reducing the amount reaching the hypothalamus and hence diminishing its hypothermic effect. Although rivastigmine also attaches to plasma cholinesterase, neither the fall in temperature nor the enzyme inhibition in the hypothalamus differed between ovariectomised and intact females, even when a dose that gave a submaximal effect was given. This may be because rivastigmine has a similar affinity for acetylcholinesterase and butyrylcholinesterase (Weinstock, 1999), while that of DFP is much greater for the plasma enzyme (Lim et al., 1989).

The increase by orchidectomy of the effects of rivastigmine on body temperature and on cholinesterase inhibition in the brain of males was restored to those in intact rats by testosterone. This hormone also significantly reduced the hypothermic effect and brain enzyme inhibition induced by rivastigmine in ovariectomised females. However, in gonadectomised males and females, testosterone increased the inhibition by rivastigmine of plasma cholinesterase, although it had no effect on basal enzyme activity. The findings suggest that testosterone may reduce the entry of rivastigmine into the brain, thereby making more rivastigmine available to inhibit the enzyme in blood. We were unable to address this question directly because sex differences in brain cholinesterase inhibition were only seen at doses up to 1.5 mg/kg (Wang et al., 2000), and the brain levels achieved at these doses were below the limit of detection by mass spectrometry (Kosasa et al., 1999). However, we were able to show that testosterone reduces the brain levels and hypothermic effect of another cholinesterase inhibitor, tacrine (Wang and Weinstock, 2001). An interference by testosterone with the access of rivastigmine to its site of action in the brain explains why cholinesterase inhibition was smaller in intact males than in females and also why orchidectomy abolished the sex difference. However, it does not explain why a considerable sex difference still remained in the hypothermic effect of the drug after removal of the testicular hormone. This may be due to an additional action at the level of the receptors subserving temperature control.

The hypothermia induced by rivastigmine was entirely blocked by scopolamine (Weinstock, unpublished observations), indicating that it resulted from activation of muscarinic receptors, probably in the preoptic area of the hypothalamus (Gordon, 1994). Rivastigmine does not have direct agonist activity so its effect on temperature is due an accumulation of acetylcholine resulting from inhibition of cholinesterase. In an attempt to study in more detail the possible modulation by testosterone of the interaction between acetylcholine and the muscarinic receptors involved in temperature regulation, we used pilocarpine, which readily enters the central nervous system. Pilocarpine also caused a greater fall in temperature in females than in males but the difference was significantly less than that after rivastigmine. Like that of rivastigmine, the effect of pilocarpine in males was increased by orchidectomy and restored to control levels by testosterone. However, in contrast to rivastigmine, testosterone had no effect on the hypothermia induced by pilocarpine in females.

These findings raise the possibility that testosterone has two actions that limit the hypothermic effect of rivastigmine

in males. It reduces the amount of drug reaching the enzyme in the brain, thereby diminishing the extent of enzyme inhibition. This could occur by genomic or membrane mechanisms in brain areas controlling cardiovascular function (Stumpf, 1990), or in blood vessels, which have receptors for testosterone (Lin et al., 1981). The hormone may also interfere with the interaction between acetylcholine or pilocarpine and its receptors. Although estrogens have been shown to alter the affinity of acetylcholine receptor agonists to binding sites in the preoptic area of the hypothalamus (Egozi et al., 1982), it is not known whether testosterone can reduce the number or affinity of such sites in males.

References

Coudray-Lucas, C., Prioux-Guyonneau, M., Tassel, A., Coq, H.M., Cohen, Y., Wepierre, J., 1981. Influence of intoxication by anticholinesterase agents on core temperature in rats: relationships between hypothermia and acetylcholinesterase inhibition in different brain areas. Acta Pharmacol. Toxicol. 49, 215–222.

Cullumbine, H., 1963. Actions at autonomic effector sites. In: Eichler, O., Farah, A., Koelle, G.B. (Eds.), Handbuch der Experimentellen Pharmakologie: Cholinesterases and Anti-cholinesterase Agents. Springer-Verlag, Berlin, pp. 515–516.

Egozi, Y., Avissar, S., Sokolovsky, M., 1982. Muscarinic mechanisms and sex hormone secretion in rat adenohypophysis and preoptic area. Neuroendocrinology 35, 93–97.

Ellman, G.L., Courtney, K.D., Anders, F., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.

Farlow, M.R., Hake, A.M., 1998. Mechanism of action and metabolism of acetyl-cholinesterase inhibitors: implications for treatment. Int. J. Geriatr. Psychopharmacol. 1 (Suppl. 1), s2–s6.

Gordon, C.J., 1994. Thermoregulation in laboratory mammals and humans exposed to anti-cholinesterase agents. Neurotoxicol. Teratol. 16, 427–453.

Gordon, C.J., Fogelson, L., 1993. Relationship between serum cholinesterase activity and the change in body temperature and motor activity in the rat: a dose–response study of diisopropyl fluorophosphate. Neurotoxicol. Teratol. 15, 21–25.

Gordon, C.J., Grantham, T.A., Yang, Y., 1997. Hypothermia and delayed fever in the male and female rat exposed to chlorpyrifos. Toxicology 118, 149–158.

Hobbiger, F., Lancaster, R., 1971. The determination of acetylcholinesterase activity of brain slices and its significance in studies of extracellular acetylcholinesterase. J. Neurochem. 18, 1741–1749.

Kosasa, T., Kuriya, Y., Matsui, K., Yamanishi, Y., 1999. Effect of donepezil hydrochloride (E2020) on basal concentration of extracellular acetylcholine in the hippocampus of rats. Eur. J. Pharmacol. 380, 101–107.

Lim, D.K., Hoskins, B., Ho, I.K., 1989. Effects of diisopropylfluorophosphate on brain acetylcholinesterase, butyrylcholinesterase, and neurotoxic esterase in rats. Biomed. Environ. Sci. 2, 295–304.

Lin, A.L., McGill, H.C., Shain, S.A., 1981. Hormone receptors of the baboon cardiovascular system: biochemical characterization of aortic cytoplasmic androgen receptors. Arteriosclerosis 1, 257–264.

Ma, T., Chambers, J.E., 1994. Kinetic parameters of desulfuration and dearylation of parathion and chlorpyrifos by rat liver microsomes. Food Chem. Toxicol. 32, 763–767.

Moser, V.C., Chanda, S.M., Mortensen, S.R., Padilla, S., 1998. Age- and sex-related differences in sensitivity to chlorpyrifos in the rat reflect developmental profiles of esterase activities. Toxicol. Sci. 46, 211–222.

Moudgil, V.K., Kanungo, M.S., 1973. Effect of age on the circadian rhythm of acetyl-cholinesterase of the brain of the rat. Comp. Gen. Pharmacol. 4, 127–130.

Overstreet, D.H., Russell, R.W., Helps, S.C., Runge, P., Prescott, A.M., 1979. Sex differences following pharmacological manipulations of the cholinergic system by DFP and pilocarpine. Psychopharmacology 61, 49–58.

Overstreet, D.H., Russell, R.W., Kerni, W., Netherton, R.A., 1981. The influence of ovariectomy on the sex-dependant effects of the anticholinesterase diisopropyl fluorophosphate. Psychopharmacology 74, 391–392.

Peskind, E.R., Raskind, M.A., Wingerson, D., Pascualy, M., Thal, L.J., Dobie, D.J., Wilkinson, C.W., 1996. Hypothalamic–pituitary–adrenocortical axis responses to physostigmine: effects of Alzheimer's disease and sex. Biol. Psychiatry 40, 61–68.

Rosler, M., Anand, R., Cicin-Sain, A., Gauthier, S., Agid, Y., Dal-Bianco, P., Stahelin, H.B., Hartman, R., Gharabawi, M., 1999. Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial. Br. Med. J. 318, 633–638.

Sen, A.P., Bhattacharya, S.K., 1991. Thermic response of selective muscarinic agonists and antagonists in rat. Indian J. Exp. Biol. 29, 131–135.

Stumpf, W.E., 1990. Steroid hormones and the cardiovascular system: direct actions of estradiol, progesterone, testosterone, gluco- and mineralcorticoids, and soltriol [vitamin D] on central nervous regulatory and peripheral tissues. Experientia 46, 13–25.

Wang, R.-H., Weinstock, M., 2001. Steroid hormones mediate sex differences in brain levels of tacrine and its hypothermic effect in the rat. Neuropharmacology 41, 887–984.

Wang, R.-H., Bejar, C., Weinstock, M., 2000. Sex differences in the effect of rivastigmine on brain cholinesterase activity and cognitive function in rats. Neuropharmacology 39, 497–506.

Weinstock, M., 1999. Selectivity of cholinesterase inhibition: clinical implications for the treatment of Alzheimer's disease. CNS Drugs 12, 307–323.