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Acetyl-L-carnitine reduces impulsive behaviour in adolescent rats

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Abstract The attention deficit/hyperactivity disorder (ADHD) can affect human infants and adolescents. One important feature of this disorder is behavioural impulsivity. This study assessed the ability of chronic acetyl-L-carnitine (ALC, saline or 100 mg/kg SC, plus 50 mg/kg orally) to reduce impulsivity in a validated animal model for ADHD. Food-restricted rats were tested during adolescence (postnatal days, pnd, 30–45) in operant chambers with two nose-poking holes, one delivering one food pellet immediately, and the other five pellets after a delay. Delay length was increased over days (from 0 to 80 s). Individual differences in the preference-delay curve emerged, with the identification of two distinct subpopulations, i.e. one with a nearly horizontal curve and another with a very steep (“impulsive”) slope. The impulsivity profile was slightly but consistently reduced by chronic ALC administration. Consistent results were also obtained with methylphenidate (MPH, saline or 3 mg/kg IP twice daily). Impulsive rats exhibited a lower metabolite/serotonin (5HIAA/5HT) ratio in the medial frontal cortex (MFC) and lower noradrenaline (NA) levels in the MFC and cingulate cortex (CC) when compared with the other subgroup. The ALC treatment increased NA levels in the CC and the 5HIAA/5HT ratio in both CC and MFC.

Present data suggest that ALC, a drug devoid of psychostimulant properties, may have some beneficial effects in the treatment of ADHD children.

Keywords Acetyl-L-carnitine · Methylphenidate · Impulsivity · Adolescence · SHR · ADHD

Introduction

It is well known from clinical practice that only a portion of psychiatric patients respond positively to a given drug therapy, and attention deficit/hyperactivity disorder (ADHD) actually seems to have heterogeneous origins (Johansen et al. 2002; Solanto 2002). These considerations derive from the notion that heterogeneous neurobiological alterations may lead to similar observed symptoms. For instance, ADHD is characterized by difficulties in inhibitory control and by impulsivity (Solanto et al. 2001; Solanto 2002). Several pathogenetic hypotheses have been proposed, including: (1) dysfunctions in the mesolimbic system or its inhibitory regulation by the pre-frontal cortex (PFC) (Johansen et al. 2002); (2) altered overproduction and pruning of dopamine (DA) receptors in the PFC (Andersen and Teicher 2000); and (3) altered serotonergic and/or noradrenergic metabolism (Oades 2002). Specifically, some symptoms of impulsivity could be ascribed to an altered serotonergic function (Linnoila et al. 1983; Soubrie 1986). Peripheral levels of the 5-HIAA serotonin metabolite are increased (Castellanos et al. 1994) and the dopamine-metabolite/serotonin-metabolite ratio is lower (Oades 2002) in ADHD versus control children, suggesting a hyperactive serotonergic system. Consistently, an elevated serotonergic function in rat PFC correlates with impulsivity (Puumala and Sirvio 1998; Dalley et al. 2002).

The spontaneously hypertensive rat strain (SHR) is a validated model of ADHD (Sagvolden et al. 1993; Mook and Neuringer 1994; Aspide et al. 2000; Sadile 2000; Sagvolden 2000; Papa et al. 2002; Russel 2002). In particular, there are considerable advantages in the use of adolescent, rather than adult, SHRs. In a series of studies

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aimed at characterizing the transitional stage of adolescence, we adopted an operant protocol for impulsive behaviour, based on a preference between either immediate-but-small or large-but-delayed food reinforcement (Thiebot et al. 1985; Evenden and Ryan 1996, 1999; Bizot et al. 1999). Two separate subpopulations were evidenced within adolescent SHR, whose preference-delay curve was either nearly horizontal or very steep (Adriani et al. 2003). According to the specific literature (Evenden and Ryan 1996, 1999), the steepness of the preference-delay curve is a reliable index of impulsivity. Hence, when adolescent SHR are tested with this protocol for intolerance-to-delay, one subpopulation appears to be very impulsive, whereas the other does not seem to pay attention to experimental contingencies, and is apparently not sensitive to delay (Adriani et al. 2003).

Methylphenidate (MPH) decreases impulsivity and increases sustained attention in humans (Ward et al. 1997). However, psychostimulant drug use is not without side effects and problems (Klein-Schwartz 2002; Rapport and Moffitt 2002). Great effort is devoted to the identification of novel non-psychostimulant agents, and acetyl-L-carnitine (ALC) has been proposed as a possible alternative. ALC is an energy reservoir (Aureli et al. 1998), which is converted through deacetylation to carnitine. The latter is a carrier of long-chain fatty acids, which are important for brain maturation and functioning (Salvati et al. 2000). ALC may increase the reservoir of activated acetyl groups, which are involved in the reacylation of membrane phospholipids (Virmani et al. 1995). ALC may prevent cellular energy deficits and limit the formation and escape of superoxide radicals from mitochondria (Hagen et al. 2002; Virmani et al. 2002; Beal 2003). ADHD comorbidity includes disorders of fatty acid metabolism (Richardson et al. 2000), their plasma concentration being decreased in ADHD patients (Stevens et al. 1995). ALC administration has been shown to modulate positively aggressive behaviour and attentional problems of ADHD children (Van Oudheusden and Scholte 2002), to reduce hyperactivity in fragile-X boys (Torrioli et al. 1999), and to improve cognitive performance in rats (Caprioli et al. 1990, 1995; Ghirardi et al. 1992).

The aim of the present study was to characterize the efficacy of ALC in adolescent SHR animals by using the intolerance-to-delay paradigm. A relief of ADHD-like symptoms in the animal model would predict its clinical efficacy in ADHD patients. Further, we aimed to characterize neurobiological parameters in brain samples from ALC-treated SHR animals, in order to add novel and useful information to the specific literature.

Materials and methods

The experimental protocols were approved by the competent institutional authorities and are in close agreement with European Community Directives and with the Italian law. All efforts were made to minimize

animal suffering, to reduce the number of animals used, and to use alternatives to in vivo testing.

Subjects, breeding, and rearing conditions

Twenty pairs of sibling male rats, belonging to the SHR strain, were purchased from a commercial breeder (Charles River Italia). Within each sibling pair, one rat was assigned to the drug-treated group, and the other rat to the control group (split-litter). Animals were 21 days old upon arrival, and were housed in an air-conditioned room (temperature $21\pm 1^\circ\text{C}$, relative humidity $60\pm 10\%$), with a 12-h light-dark cycle (lights on at 8.00 a.m.). Two non-sibling rats were housed in each Plexiglas cage, according to drug assignment. Water and food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, Italy) were available ad libitum. Animals were given at least 10 days of recovery before the experiment started, and were tested between 30 and 45 days of age.

Experimental schedule

Two days before the schedule started, animals were food-restricted, in order to increase their motivation to work for food delivery. Animals were put daily (between 10.00 a.m. and 4.00 p.m.) in computer-controlled operant chambers, provided with two nose-poking holes (diameter 30 mm), a chamber light, a feeder device with a magazine where pellets were dropped, and a magazine light (Coulbourn Instruments, USA). The nose-poking in either hole was detected by a photocell and was recorded by the computer. Food delivery was contingent upon animals' nose-poking, depending on the specific phase of the schedule (training phase or test phase). After a 30-min session, animals were returned to their home cage, where they were given standard chow (approximately 6 g each). This procedure kept the animals at two-thirds of their standard weight (see Table 1). Despite a reduced rate of body weight gain, these growing animals were completely healthy and expressed a playful behavioural repertoire.

During the training phase (1 week), nose-poking in one of the two holes (called "immediate and small" hole, H1) resulted in the delivery of one pellet (45 mg, BioServ, USA) of food, whereas nose-poking in the other hole

Table 1 Mean (\pm SEM) body weight (g) before and during the operant-behaviour schedule in control animals

Schedule	Age (pnd)	Weight
Day-6	26	54.3 \pm 1.5
Day-4	28	63.5 \pm 1.7
Day-2	30	74.9 \pm 1.8
Day+1	33	64.7 \pm 1.7
Day+3	35	58.3 \pm 1.5
Day+5	37	66.8 \pm 1.4
Day+8	40	71.5 \pm 1.3
Day+10	42	77.9 \pm 1.3
Day+12	44	83.4 \pm 1.2

(“large and delayed” hole, H5) resulted in a 1-s flash of the chamber light and the delivery of five pellets of food. Following the food delivery, the magazine light was turned on for 25 s, during which additional nose-pokes in either hole were recorded but were without consequence (time-out). As expected, during the training period, all animals quickly developed a significant preference for the H5 hole delivering the large reinforcer. The test phase started when all animals were choosing the large reward at least 66% of times. In these conditions, the preference for H5 reached significance, namely the chance level (50%) was in the 5% tail of the normal distribution curve.

During the test phase (1 week), a delay was inserted between nose-poking in the H5 hole and the delivery of the five pellets. During this delay, the chamber light was turned on and additional nose-pokes in either hole were recorded but were without consequence (“inadequate responding”, see [Results](#)). The delay was fixed for a given daily session and increased progressively over subsequent days. All rats were expected to shift their preference from the large and delayed reward towards the immediate and small one, as the delay increased.

Drugs

Methylphenidate (ritalin; Ciba-Geigy SpA, Italy) and acetyl-L-carnitine (Sigma-Tau SpA, Italy) were dissolved in saline (SAL, NaCl 0.9%) and injected in a volume of 1 ml/200 g body weight. Sibling rats belonging to the control group were injected with saline.

Effect of methylphenidate on impulsivity

Rats without previous testing experience were tested during adolescence (from 30 to 45 days old) using the experimental schedule described above ($n=10$ per group). All animals were injected twice daily (at 8:30 a.m. and 7:30 p.m.) with either SAL or MPH (3 mg/kg IP). Operant-behaviour sessions took place at least 90 min after the first MPH injection.

Neurochemical and behavioural effect of ALC

Rats without previous testing experience were tested during adolescence (from 30- to 45-day-old) using the experimental schedule described above ($n=10$ per group). All animals were injected once daily 15 min before the start of their session, and received SAL or ALC (100 mg/kg SC). In addition, from 1 week before the start of the schedule till death, animals belonging to the drug-treated group also received ALC dissolved in the drinking water (200 mg/l). This concentration was calculated in order to provide an intake of approximately 50 mg/kg per day. Sibling rats belonging to the control group received tap water.

After behavioural testing, subjects were given ad libitum access to food. For neurochemical measures, rats were sacrificed by decapitation and their brains were quickly frozen on dry-ice and dissected. The medial-frontal cortex (MFC), cingulate cortex (CC), striatum, nucleus accumbens, hippocampus, brainstem and remaining cortex were dissected out frozen on dry ice and stored at -80°C until neurochemical assays. These areas were assessed for levels of the following neurotransmitters: noradrenaline (NA), serotonin (5-HT), dopamine (DA), and their metabolites.

Brain tissues were homogenized by sonication using a Branson sonicator (model 250, Branson, Danbury, Conn., USA) in 10 vol ice-cold 0.1 M HClO_4 . Homogenates were left at 4°C for 30 min and then centrifuged at 9000g for 10 min at 4°C . Twenty microlitres of supernatant was injected into the high performance liquid chromatograph coupled to an electrochemical detector (HPLC-ED) for the determination of NA, DA, 5-HT, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxy-indoleacetic acid (5-HIAA) essentially according to the procedure described elsewhere (Lasley et al. 1984). The HPLC-ED system utilized a 150×3.9 mm C18 Nova-Pack reverse phase column (Waters, Milano, Italy) coupled to a coulometric detector (model 5100, ESA, Chelmsford, Mass., USA) equipped with a 5011 cell. The potential setting was $E_1=+0.0$ V and $E_2=+0.37$ V. Samples were injected into a six-port valve (model 7125; Rheodyne, Berkeley, Calif., USA) via a 20 μl PEEK loop. The mobile phase consisted of 0.15 M chloroacetic acid, 0.86 mM sodium octyl sulphate, 0.1 mM Na_2EDTA , 15% CH_3OH , pH 2.75 with 5 M NaOH. Flow rate (1 ml/min) was maintained by an SP8810 pump (Spectra Physics, San Jose, Calif., USA).

Design and data analysis

Data were analysed by a split-plot ANOVA (Zorrilla 1997), where the pair of sibling subjects was the block variable. The parameter considered was the number of “adequate” visits (nose-poking resulting in the delivery of the reinforcer). According to previous results from our group (Adriani et al. 2003), data were analyzed in terms of individual differences, a procedure that has been largely validated in animal studies (Piazza et al. 1991; Hooks et al. 1994). Namely, for each control individual, the slope of the preference-delay curve was calculated across the whole testing period. All animals were divided into two subgroups on the basis of the distribution of this slope value and its median (Piazza et al. 1991; Hooks et al. 1994). Sibling pairs were hence assigned to one of two subgroups, characterized by either a flat (lower-than-median) or a steep (higher-than-median) preference-delay curve. Post-hoc multiple comparisons within a significant interaction were performed using the Tukey HSD test.

For behavioural data, three dependent variables were considered. The first variable was the hole-preference (calculated at each delay as percent visits at the H5 hole

over total H5+H1 visits). The second variable was the amount of non-reinforced responding (i.e. nose-poking during the length of the delay, when it was without scheduled consequence). The general design was a 2 drug (ALC versus SAL)×delay (0–80 s) model. Two separate analyses were performed for each of the two slope subgroups (the “non-sensitive” and the “delay-sensitive” ones), whose behavioural profile has been previously shown to differ markedly (Adriani et al. 2003). The third variable was the slope of the preference-delay curve (Microsoft Excel “slope” function, using hole-preference as the *Y*-axis data and $\log(\text{delay}+1)$ as the *X*-axis data). The general design was a 2 drug (ALC versus SAL)×2 subgroup (non-sensitive versus delay-sensitive) model.

For neurochemical data, two dependent variables were considered, namely the level of each neurotransmitter and its turnover (calculated as the metabolite/transmitter ratio). The general design was a 2 drug (ALC versus SAL)×2 subgroup (non-sensitive versus delay-sensitive) model.

Results

Effect of methylphenidate on impulsivity

In the present study, methylphenidate was used to further validate the operant paradigm and as a reference drug for ALC effects.

Within the non-sensitive subpopulation, as delay increased, the hole-preference shifted only slightly towards the H1 hole, delivering the small reinforcer [delay, $F(7,56)=12.6$, $P<0.001$]. The subjects in this group chose the H5 hole a majority of the time, even at the highest delays (see Fig. 1 left panel). Interestingly, the main effect of drug [$F(1,8)=0.533$] and the drug-by-delay interaction [$F(7,56)=1.19$] were not significant. Such a finding indicates that MPH administration had no effect on the operant-behaviour profile shown by animals of the non-sensitive subpopulation.

Within the delay-sensitive and impulsive subpopulation, the preference progressively shifted towards the H1 hole as delay increased [delay, $F(7,56)=28.5$, $P<0.001$]. The drug-by-delay interaction [$F(7,56)=2.37$, $P<0.05$] was found to be significant. This result indicates that MPH administration was able to modulate the impulsivity profile of this group. In particular, as the delay increased, the administration of MPH resulted in the maintenance of increased preference for the H5 hole (see Fig. 1 right panel). Hence, the subjects administered MPH appeared to shift much less towards the H1 hole, when compared with the SAL-injected subjects ($P<0.05$).

This profile was confirmed by the ANOVA performed on the slope data (see Table 2). The ANOVA showed a significant subgroup by drug interaction [$F(1,15)=6.48$, $P<0.05$]. Multiple comparisons revealed that no differences in slope were produced by the drug treatment within the non-sensitive subpopulation. Interestingly, SAL-injected controls within delay-sensitive and impulsive subpopulation exhibited the highest overall slope (in absolute

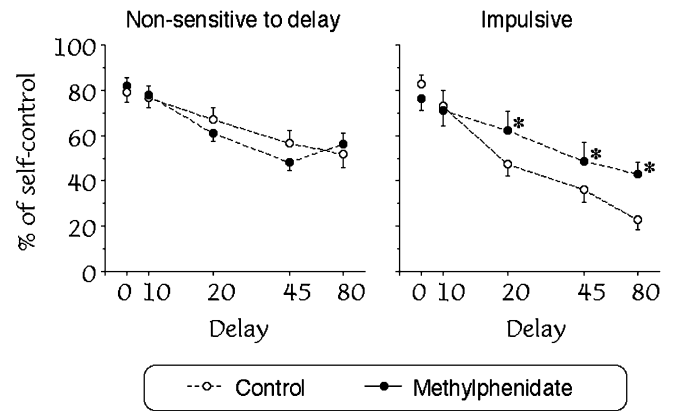


Fig. 1 Mean (\pm SEM) choice (%) of the large reinforcer, demanded by nose-poking at the H5 hole, shown by adolescent SHR during the impulsivity test. *Left panel*: no effects of MPH were found in the non-sensitive subgroup. *Right panel*: when compared to SAL-injected controls, MPH treatment increased preference for the delayed reinforcer, i.e. reduced impulsivity, within delay-sensitive (impulsive) rats. Drug effect was evident without need of linear regression and slope analysis. * $P<0.05$ between drug-treated and SAL-injected groups, within a given subpopulation ($n=9-10$)

values), suggesting a very steep preference-delay curve. Conversely, the absolute value of the slope in MPH-administered rats was generally lower than in SAL controls ($P<0.05$), indicating a flatter preference-delay curve. This set of data allows the conclusion that MPH treatment was able to reduce the prominent impulsivity profile normally shown by control subjects of the delay-sensitive and impulsive subpopulation.

The nose-poking in either hole during the course of the delay was considered an “inadequate response”, since it had no scheduled consequences. As the length of the delay increased, the nose-poking in the H5 hole was progressively reduced, whereas the nose-poking in the H1 hole increased progressively [delay, $F(6,84)=8.53$ and 5.95 , respectively, $P<0.001$]. This finding suggests that, when animals had to wait for a large reinforcer, they were unable to stay still and started demanding for the small-and-immediate reward (see, e.g. Adriani et al. 2003). Interestingly, in the absence of reliable drug effects in non-sensitive rats, MPH administration seemed to affect non-reinforced responding of delay-sensitive and impulsive rats. In particular, a tendency for increased non-reinforced responding at the H5 hole [drug, $F(1,7)=4.27$,

Table 2 Changes in the slope of the preference-delay curve, shown by adolescent non-sensitive and delay-sensitive SHR, as a consequence of drug treatments

Exp.	Treatment	Non-sensitive	Delay-sensitive
MPH	Saline	-13.87 \pm 1.77	-30.77 \pm 2.58
	Methylphenidate	-16.86 \pm 2.22	-22.01 \pm 2.98*
ALC	Saline	-10.85 \pm 2.83	-31.15 \pm 2.96
	Acetyl-L-carnitine	-14.75 \pm 2.56	-23.18 \pm 4.13*

* $P<0.05$ in post-hoc comparisons between drug-treated and saline-injected groups, within a given subpopulation

$P=0.073$], but not at the H1 one [drug, $F(1,7)=0.02$, NS], appeared upon MPH administration (data not shown). Such a finding suggests that MPH-administered animals reacted to the first increases in delay duration by nose-poking at the H5 hole, rather than shifting immediately toward the H1 hole.

Behavioural effects of acetyl-L-carnitine

As the delay increased, non-sensitive animals exhibited only a slight shift towards the H1 hole [delay, $F(7,63)=13.1$, $P<0.001$], since they chose the H5 hole a majority of the time, even at the longest delays (see Fig. 2 left panel). Not significant effect were found for drug [$F(1,9)=0.428$] and for drug-by-delay interaction [$F(7,63)=1.04$], suggesting that ALC exposure had no effect on this subpopulation.

For the delay-sensitive and impulsive subpopulation, as the delay increased, the rats' preference shifted progressively towards the H1 hole [delay, $F(7,63)=33.3$, $P<0.001$]. The main effect of drug and also the drug-by-delay interaction were not significant [$F(1,9)=2.23$ and $F(7,63)=1.32$, respectively]. However, some tendency for a statistically significant ALC effect was revealed by multiple comparisons. In particular, at the longest delay, the exposure to ALC resulted in a higher preference for the H5 hole than SAL controls ($P<0.05$). Hence, subjects receiving ALC treatment tended to shift slightly less towards the H1 hole, when compared to the SAL control subjects (see Fig. 2 right panel).

This tendency was confirmed by the ANOVA performed on the slope data (see Table 2) where a significant subgroup-by-drug interaction [$F(1,18)=5.99$, $P<0.05$] was found. Multiple comparisons revealing no differences in slope were produced by the drug treatment within the non-sensitive subpopulation. Conversely, ALC administration

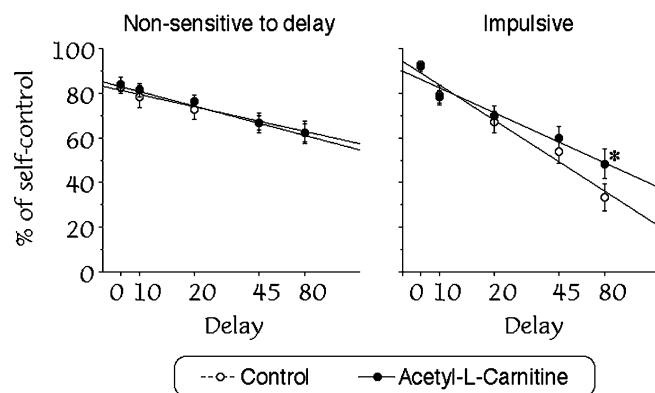


Fig. 2 Mean (\pm SEM) choice (%) of the large reinforcer, demanded by nose-poking at the H5 hole, shown by adolescent SHRr during the impulsivity test. The slight drug effect became more evident after linear regression and slope analysis. *Left panel*: no effects of ALC were found in the non-sensitive subgroup. *Right panel*: when compared with SAL-injected controls, ALC treatment reduced the steepness of the curve, i.e. reduced impulsivity, within delay-sensitive (impulsive) rats. * $P<0.05$ between drug-treated and SAL-injected groups, within a given subpopulation ($n=10$)

consistently lowered the absolute value of the slope in rats within delay-sensitive and impulsive subpopulation ($P<0.05$). Such a profile indicates a flatter preference-delay curve, and suggests that ALC exposure was associated with a slight but significant reduction in the elevated levels of impulsivity.

As expected for non-reinforced responding, the nose-poking in the H5 hole was progressively reduced, whereas the nose-poking in the H1 hole increased progressively [hole \times delay, $F(7,63)=29.7$, $P<0.001$] as the length of the delay increased. No reliable effects of ALC exposure were found in either subpopulation.

Neurochemical effects of acetyl-L-carnitine

No significant or reliable differences were found in DA levels and utilization in the MFC and CC among experimental groups.

No significant differences among groups were found for NA turnover in MFC and CC. No significant difference in 5-HT levels in MFC and CC were found among groups.

Noradrenaline (NA) levels in the MFC The ANOVA revealed a main effect of the subpopulation factor [$F(1,18)=7.40$, $P<0.05$]. Specifically, animals of delay-sensitive and impulsive subpopulation showed lower NA levels when compared to the non-sensitive subpopulation (see Fig. 3, upper left panel). ALC exposure had no effects on NA levels.

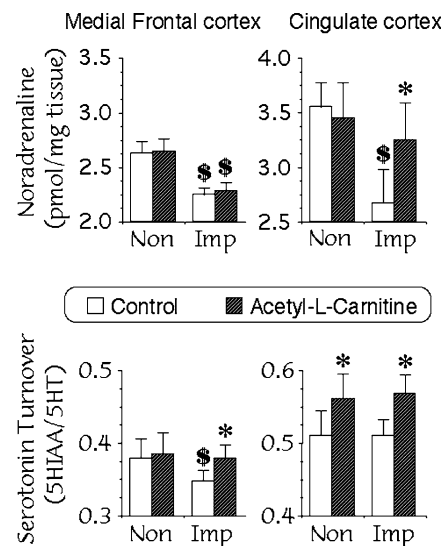


Fig. 3 Neurochemical assessment in SHRr of a non-sensitive (*Non*) and a delay-sensitive, impulsive (*Imp*) subpopulation, as a function of ALC exposure during adolescence. *Upper panels*: mean (\pm SEM) levels of noradrenaline (pmol/mg tissue) in the medial-frontal (*left panel*) and cingulate (*right panel*) cortex. *Lower panels*: mean (\pm SEM) serotonin turnover, as measured by the 5HIAA/5HT ratio, in the medial-frontal (*left panel*) and cingulate (*right panel*) cortex. Post-hoc comparisons: $^{\S}P<0.05$ between delay-sensitive and non-sensitive subjects; * $P<0.05$ between drug-treated and saline-injected groups, within a given subpopulation

Noradrenaline (NA) levels in the CC The ANOVA showed a significant drug by subpopulation interaction [$F(1,15)=5.37, P<0.05$]. Specifically, SAL-injected control animals of delay-sensitive and impulsive subpopulation showed lower NA levels, when compared to the corresponding non-sensitive group. Moreover, ALC exposure within delay-sensitive and impulsive subpopulation resulted in a significant increase in NA levels over SAL controls (see Fig. 3, upper right panel).

Serotonin utilization (5-HIAA/5-HT) in the MFC The ANOVA showed a significant main effect of drug [$F(1,18)=6.22, P<0.05$] and a significant drug by subpopulation interaction [$F(1,18)=3.31, P<0.05$]. Post-hoc comparisons revealed that control animals of delay-sensitive and impulsive subpopulation showed a lower 5HIAA/5-HT ratio, when compared with the corresponding non-sensitive group. Moreover, within delay-sensitive and impulsive subpopulation, ALC exposure resulted in a significant increase of the 5HIAA/5-HT ratio ($P<0.05$), when compared with SAL controls (see Fig. 3, lower left panel).

Serotonin utilization (5-HIAA/5-HT) in the CC The ANOVA revealed a main effect of the drug factor [$F(1,15)=4.37, P<0.05$]. Specifically, ALC-exposed animals of both subpopulations showed increased 5HIAA/5-HT ratio, when compared to the control groups (see Fig. 3, lower right panel). No significant and reliable differences were found between the two subpopulations.

Discussion

The main findings of the present study can be summarized as follows:

- (1) ALC administration somewhat reduced impulsivity in one subgroup of SHR subjects. ALC effects were only found within the subpopulation that was sensitive to delay, with no changes at all in non-sensitive rats.
- (2) When compared to non-sensitive rats, the delay-sensitive and impulsive subpopulation showed lower basal NA levels in both the CC and the MFC, as well as a lower basal 5-HIAA/5-HT ratio in the MFC. Chronic ALC exposure significantly increased the NA levels in the CC and the 5-HIAA/5-HT ratio in both the CC and MFC of impulsive rats.

The SHR strain has been validated as an animal model of ADHD in tests for operant and attentional behaviour (Aspide et al. 2000; Sagvolden 2000; see, however, Ferguson and Cada 2003). One advantage of testing the SHR during adolescence is to minimize the effects of elevated blood pressure on behavioural responsiveness. In addition, a temporary rearrangement in functional dopaminergic parameters occurs during adolescence (Stamford 1989; Teicher et al. 1995; for behavioural correlates, see Laviola et al. 2003; Tirelli et al. 2003). Recently, two subpopulations were evidenced within the adolescent SHR (Adriani et al. 2003). Accordingly, in the present study, a

first subgroup showed a very steep preference-delay curve, a classical index of enhanced impulsivity (Evenden and Ryan 1996, 1999). Conversely, a second subgroup showed little or no preference-shift, in that animals did not seem to pay attention to experimental contingencies and were apparently not sensitive to delay (Adriani et al. 2003).

The effect of psychostimulants in the control of self-inhibition is controversial. Some authors indicate a decrease in self-control following cocaine (Logue et al. 1992) and amphetamine (Evenden and Ryan 1996), whereas increased self-control following amphetamine (Wade et al. 2000) or methamphetamine (Richards et al. 1999) is also reported. Differences in the operant protocols may probably account for this discrepancy. Similarly, the effect of psychostimulants in humans is also controversial. On the one hand, these drugs induce arousal, euphoria and reward, and schizophrenia-like symptoms at high doses. Conversely, the same drugs reduce hyperactivity, inattention and impulsivity in ADHD children (Ward et al. 1997). This paradox is often solved by admitting a rate-dependent effect (Glick and Milloy 1973).

In the present study, MPH administration was able to reduce impulsivity in adolescent SHRs. Such findings further validate both the intolerance-to-delay paradigm for the study of impulsivity and the use of the SHR as an animal model of ADHD children (see also Paule et al. 2000; Ueno et al. 2002). As for possible mechanisms, MPH administration seems to activate the PFC and the striatum in ADHD children (Vaidya et al. 1998). In addition to its well-known effects on DA system (see Papa et al. 2002; Russell 2002; Yang et al. 2003), MPH also increases NA release (Kuczenski and Segal 2002) and 5-HT turnover (Kuczenski et al. 1987) in rats. In the present study, no significant effects of MPH or ALC were found for the non-sensitive subgroup. Of course, in the subgroup that was apparently not sensitive to delay, the lack of drug effects is devoid of predictive value for drug-induced effects on behavioural parameters.

ALC effects on impulsive behaviour and neurochemical parameters

ALC treatment affected the operant-behaviour performance, since the steepness of the preference-delay curve (i.e. "impulsivity") was consistently and reliably reduced. Compared with MPH, the magnitude of the effect was somewhat slight, in that a tendency towards higher self-control levels in the ALC-treated group emerged only at the longer delay. However, when a linear regression and slope analysis were performed, drug effects were statistically significant. This profile is consistent with recent clinical data, reporting some beneficial effects of ALC on ADHD children (Torrioli et al. 1999; Van Oudheusden and Scholte 2002).

As for neurochemical parameters, impulsive rats had lower basal NA levels and a lower 5-HIAA/5-HT ratio, compared with non-sensitive subjects. So far, the serotonergic impairment in impulsivity is poorly understood.

Consistently, reduced central 5-HT activity predisposes to impulsive tendencies in sustained-attention tasks (Harrison et al. 1997a,b) and in delay-of-reward choice procedures (Wogar et al. 1993; Mobini et al. 2000). Recently, however, elevated serotonergic functions in rat's PFC have been associated with impulsivity in the five-choice serial reaction time task (Puumala and Sirvio 1998; Dalley et al. 2002). Changes in age, strain, and in operant-task demands might of course explain the observed differences. Differently from 5-HT, the role of forebrain NA is to selectively focus the attention, and to maintain it in the presence of arousal, stress, or distracting conditions (Carli et al. 1983; Robbins 1984, 2002). Altered NA activity has been reported in ADHD children (Halperin et al. 1997), which may be unable to tune in/out relevant stimuli in rapidly changing situations (Oades 2002). Animals with 6-OHDA-induced NA lesions are actually inattentive in highly arousing conditions (Carli et al. 1983). The individual differences in basal NA levels, found in the present study, may account for the emergence of a non-sensitive subgroup of rats.

ALC exposure during adolescence produced a significant increase in both NA and 5-HT parameters in impulsive rats, with little or no effect on non-sensitive animals. As a whole, ALC effects on neurochemical parameters seem to mirror those on the behavioral performance, in that they were evident only in delay-sensitive subjects. It might be suggested that reduced impulsivity depends to some extent on these NA and 5-HT changes. ALC may act by improving energy and phospholipid metabolisms (Aureli et al. 1998), and may protect neuronal function by action at the mitochondrial level (Hagen et al. 2002; Virmani et al. 2002; Beal 2003). Being a donor of acetyl groups, ALC is classically reported to enhance acetylcholine release (Imperato et al. 1989) and to facilitate the neuronal response to acetylcholine (Tempesta et al. 1985). Recently, a more specific action on neurotransmission has been proposed in rats, with ALC-induced increase of glucose utilization in the raphe, the locus coeruleus, and in limbic areas (Ori et al. 2002), increased DA and 5-HT release from both nigrostriatal (Harsing et al. 1992) and mesolimbic neurons (Tolu et al. 2002), as well as facilitated neuronal response to 5-HT (Tempesta et al. 1985). It is interesting to note that CC-lesioned rats are highly impulsive (Muir et al. 1996). Hence, the ALC-induced elevation of both NA levels and 5-HT utilization in the CC might contribute to the reduced impulsivity observed in the present study.

Conclusion

Repeated treatments with psychostimulants during adolescence might represent a risk factor for the subsequent development of drug abuse in ADHD patients (Schenk and Davidson 1998; Robinson and Berridge 2000; Brandon et al. 2001; see, however, Andersen et al. 2002). The discovery of new therapeutic agents, devoid of psychostimulant activity, would be very important to reduce side

effects in the treatment of ADHD children. Present findings are relevant to both preclinical literature and clinical practice.

We showed within adolescent SHR rats that impulsive subjects respond positively to a MPH treatment, further validating the impulsivity paradigm and the animal model of ADHD. Interestingly, chronic treatment with ALC (a non-psychostimulant drug) also reduced impulsive behaviour in this animal model, thus suggesting a possible clinical benefit in the therapy of ADHD children. Finally, our present findings support the notion that impulsive behaviour may arise from individual differences in frontostriatal 5-HT and NA systems.

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