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# Genotypic Variation in the Dopaminergic Inhibitory Control of Striatal and Hippocampal Cholinergic Activity in Mice

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DURKIN, T. P., H. HASHEM-ZADEH, P. MANDEL, J. KEMPF AND A. EBEL. *Genotypic variation in the dopaminergic inhibitory control of striatal and hippocampal cholinergic activity in mice.* PHARMACOL BIOCHEM BEHAV 19(1) 63–70, 1983.—Genotypic variation in dopaminergic-cholinergic interactions in striatum and hippocampus has been investigated in the C57BL/6 and BALB/c mouse strains. Acute treatment *in vivo* with increasing doses of haloperidol provided behavioural evidence for significant strain-dependent variation in neuroleptic sensitivity as assessed by catalepsy scores with C57BL/6 mice being the more sensitive strain. Measures of the kinetics of high affinity choline uptake in crude synaptosomal preparations of striatum and hippocampus from haloperidol treated mice indicated parallel strain variations for the dose-related activation (dis-inhibition) of cholinergic activity in these regions. The relative involvements of the D1-D2 receptor subpopulations in these effects was investigated using the D2 selective antagonist sulpiride. Sulpiride was also observed to produce similar strain-dependent activation of cholinergic activity in both brain regions whereas behavioural effects were not marked and catalepsy absent in both strains. Comparative *in vitro* studies on the sensitivity of the dopamine stimulated adenylate cyclase from striatal membranes of both strains failed to reveal significant differences. Genotypic variation in agonist sensitivity was explored using apomorphine. While BALB/c mice exhibited significantly greater sensitivity than C57BL/6 regarding dose effects on stereotypy and rearing, apomorphine was found to be without significant effect in either strain regarding striatal or hippocampal high affinity choline uptake kinetics. These results constitute additional evidence for the proposal that cholinergic activity in striatum and hippocampus is tonically inhibited by dopaminergic mechanisms mediated by the D2 receptor sub-population and further that their density and/or functional activity exhibits significant genotypic variation.

Inbred mouse strains  High affinity choline uptake  Dopamine receptors  Neuroleptics  Apomorphine  Striatum  Hippocampus  Genotypic variation

A MAJOR research strategy for the investigation of the neurochemical bases underlying behavioural expression has been provided by comparative studies on neurotransmitter activity in different regions of the brains of inbred mouse strains which show genotypic and often marked differences in some aspects of their behaviours. Results from these types of studies have established that the concentration or turnover rates of several neurotransmitters as well as the specific activities of their synthesising or degradative enzymes and receptor densities frequently show genotypic variation and regional selectivity in mouse brain [2, 6, 8, 12, 14, 39, 46]. These observations raise the possibility that some of the variations in regional neurotransmitter activity are due to primary differences in the genetic mechanisms controlling the expression of certain neurotransmitter systems whereas others may be secondary to the resulting imbalance in neurotransmitter interactions. One major consequence is that the observed strain differences in behavioural expression, sensitivity to certain psychotropic drugs and, perhaps, in man susceptibility to certain central disorders may have as their origin such genetically determined differences in the expression of certain neurotransmitters and their interaction with other systems.

We have observed in previous studies [13] that presynaptic cholinergic activity, as measured by the Vmax of the sodium-dependent high affinity choline uptake mechanism in crude synaptosomal fractions of striatum and hippocampus shows significant genotypic variation, being greater in both brain regions of C57BL/6 mice than in mice of the BALB/c strain. Recent evidence supports the concept of a tonic inhibitory control mediated by A9 dopaminergic afferents of the activity of the cholinergic striatal interneurones and by A10 afferents of the neurones of the cholinergic septo-hippocampal pathway [1, 10, 17, 32, 33, 37]. Comparative

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studies on central dopaminergic activity in the BALB/c, CBA/J and C57BL/6 mouse strains have revealed significant strain differences in the number of midbrain dopaminergic neurones, striatal tyrosine hydroxylase specific activities [4,15] and the sensitivity of the dopamine stimulated adenylate cyclase of striatal homogenates [11, 31, 44]. Furthermore major strain differences in the density of striatal dopamine receptors as measured by ($^3$H) spiroperidol or ($^3$H) (±)2-amino-6,7-dihydroxytetrahydronapthalene (ADTN) binding [6,39] have also been observed.

In the present study we have investigated this genotypic variation in central dopaminergic neurotransmission and its functional impact on dopaminergic-cholinergic interactions by measuring changes in the kinetics of the sodium-dependent high affinity choline uptake mechanism as an index of changes in the activity of cholinergic neurones in the striatum and hippocampus following the *in vivo* treatment of C57BL/6 and BALB/c mice with different classes of dopaminergic drugs. Using dopaminergic antagonists with differing specificities towards D1 type (adenylate cyclase linked) or D2 type receptors we attempted (1) to quantify strain differences in neuroleptic sensitivity by measuring the amplitude, dose-dependence and time course of the activation (dis-inhibition) of cholinergic activity resulting from the acute blockade of the tonic dopaminergic inhibition in these brain regions; (2) to determine whether the tonic dopaminergic inhibition is mediated via D1 or D2 type receptors [25,41] and whether their relative densities of functional activity show significant strain differences. In order to pursue this latter point we also conducted comparative studies of the *in vitro* sensitivity of the dopamine stimulated adenylate cyclase system in washed striatal membranes from both mouse strains. Genotypic variation in behavioural and neurochemical sensitivity to dopamine agonists was probed using acute treatments with apomorphine in dose ranges producing stereotyped behaviour and rearing since this strategy has been considered [30] as a convenient means to assess the stimulation of striatal dopamine receptors in mice.

## METHOD

### *Animals and Drugs*

Male mice of the BALB/c ORL and C57BL/6 ORL strains were obtained at the age of six weeks from the "Centre d'Elevage du C.N.R.S." Orléans la Source, France. They were group-housed in controlled light and temperature conditions with unlimited access to standard rodent pellets and water. Mice were aged between 8 to 12 weeks for these experiments in which animals of the same age from both strains were compared. All experiments were started after exposure of 5 to 6 hr of light.

Haloperidol (HALDOL, Laboratoires LeBrun, Paris), sulpiride (DOGMATIL, Laboratoires Delagrange, Paris), apomorphine hydrochloride (Laboratoires Aguettant, Lyon) were used and diluted just before use in physiological saline. Following systemic injection mice were replaced in standard cages, grouped according to treatment for behavioural observation.

### *Behavioural Observation*

Locomotor behaviour following the injection of either haloperidol, sulpiride or vehicle was evaluated by direct observation for 20 seconds every 5 minutes for up to one hour. Activity was rated on a four point scale by two independent observers with 4 being equivalent to normal locomotion and lower ratings indicating progressively reduced activity. Catalepsy (rating 1) was reliably tested if not already spontaneously present by a slight backwards pull on the tail with the mouse head down on a slightly sloping surface. A minimum criterion of 3 seconds immobility of all four paws was used to define catalepsy and the total seconds immobile during each observation period measured.

Apomorphine induced stereotypic behaviour was also evaluated by direct observation for 20 seconds every 5 minutes for up to one hour. A rating scale similar to that described in [39] was used where (1) is equivalent to reduced locomotor activity, (2) reduced locomotor activity with intermittent sniffing, (3) continual sniffing maintaining some whole body movement, (4) upright head posture and intermittent sniffing, (5) upright head posture (arched back) and continual sniffing, (6) extreme upright posture with continual sniffing. For both series of behavioural observations good inter-observer agreement was achieved ($r=0.87$). Statistical analysis of strain differences in these behaviours was conducted for the second 30 min period of observation in order to circumvent problems arising from differences in the latency of action of these drugs.

### *Sample Preparation*

Animals were sacrificed by a modification of the near freezing method by plunging the head region into liquid nitrogen for 1 sec followed by immediate decapitation. The brain was removed and placed onto a glass plate cooled by liquid nitrogen vapour and the striatum or hippocampal formation immediately dissected and placed at 0–2°C. Dissection was conducted as rapidly as possible consistent with sample purity to avoid post-mortem changes to high affinity choline uptake [26].

### *Biochemical Analysis*

Sodium-dependent high affinity choline uptake kinetics were measured in aliquots of resuspended crude synaptosomal (P2) pellets using a procedure based on that of Atweh *et al.* [3] and described by us previously [13].

For comparative *in vitro* studies on the dopamine sensitive adenylate cyclase, washed striatal particulate fractions were used and prepared as follows. Striata were dissected from each strain and placed at 0–2°C. Tissue was homogenised by hand in 50 volumes of 1 mM Tris-maleate buffer pH 7.5 containing 1 mM ethyleneglycol bis (β-amino ethyl ether) N,N'tetraacetic acid (EGTA) using 10 strokes of a Dounce glass-glass homogeniser. The homogenate was stirred continuously at 0–2°C for 10 min to ensure complete lysis and then centrifuged at 50,000 × g for 30 min. The supernatant was eliminated and the pellet resuspended in the original volume of 10 mM Tris-maleate buffer pH 7.5 containing 1 mM EGTA. Following a second centrifugation at 50,000 × g for 30 min the washed pellet was rehomogenised in 1 ml of fresh 10 mM Tris-maleate buffer pH 7.5 and 1 mM EGTA using a Polytron (position 5) for 15 seconds. The washed particulate fractions were stored at −80°C until use.

The activity of adenylate cyclase (ATP pyrophosphate-lyase (cyclizing) E.C.4.6.1.1) was determined in aliquots of the thawed striatal particulate fractions by measuring the conversion of α-($^{32}$P) ATP to ($^{32}$P) cyclic AMP. Reactions were conducted in a final volume of 100 μl containing (final concentrations) 50 mM Tris-maleate buffer pH 7.5 mM cyclic AMP, 2 mM magnesium chloride, 1 mM theophylline,

500 $\mu$M EGTA, 0.1 mg/ml creatine kinase, 10 mM creatine phosphate, 0.5 mM ATP (approximately 4 million cpm of $\alpha$-($^{32}$P)ATP), 10 $\mu$M GTP and appropriate drugs. Reaction mixtures were made up, but without the ATP substrate and an aliquot of the washed striatal particulate fraction (containing 5 $\mu$g of protein) added. Following pre-incubation at 30°C for 2 min reactions were initiated by the addition of $\alpha$-($^{32}$P) ATP and allowed to continue for 5 min. The incubations were terminated by the addition of 100 $\mu$l of a solution containing 50 mM Tris-maleate buffer pH 7.5, containing 5 mM ATP and 10% sodium dodecyl sulphate. After addition of 20,000 cpm of ($^{3}$H) cAMP as an internal standard to quantitate recovery (routinely 80%) sample volumes were adjusted to 1 ml using distilled water and cyclic AMP was separated from the ATP substrate by the method of Salomon *et al.* [34] using successive Dowex and alumina columns. Radioactivity associated with the ($^{32}$P) cyclic AMP was measured in a liquid scintillation counter and after applying correction for recovery, expressed as pmoles of cyclic AMP formed per minute and per milligram of protein using the known specific radioactivity of the $\alpha$-($^{32}$P) ATP precursor. Protein content was determined in every sample by the method of Lowry *et al.* [27] using bovine serum albumin as standard. The apparent Michaelis constants for high affinity sodium dependent choline uptake $K_T$ and Vmax were obtained from plots of V against V/S or from double reciprocal plots and the lines of best fit determined by linear regression analysis. Statistical analysis of neurochemical data was conducted using Student's *t*-test whereas strain comparisons of behavioural data were analysed using non-parametric statistics (Mann-Whitney U test) for small samples [40].

## RESULTS

### *Strain Comparison of the Acute Effects of Haloperidol*

Groups of mice from both strains were treated with doses of haloperidol of 0.5, 1 and 2 mg/kg IP or vehicle for periods of up to one hour.

Highly significant behavioural differences in strain sensitivity to these doses of haloperidol were observed over this relatively short time period. Mice of the C57BL/6 strain exhibited catalepsy at each dose following a latency of 5 to 10 min and the severity (time immobile) was observed to increase with dose. Cumulative group means (seconds immobile) during the second 30 min observation period were 5.3 sec for the 0.5 mg/kg dose and 8.9 for 1.0 mg/kg. Identical injections of mice of the BALB/c strain, however, resulted only in lowered activity ratings for doses of 0.5 and 1 mg/kg with catalepsy being observed only following 2 mg/kg doses in this strain with a minimum latency of 20 min. Even at this dose BALB/c mice were observed to exhibit phasic catalepsy and ratings of time spent immobile were equivalent or even inferior to those observed in C57BL/6 mice at 0.5 mg/kg doses during the second 30 min period. Cumulative group means (seconds immobile) for the second 30 min observation period in mice treated with 2 mg/kg haloperidol were C57BL/6 12.6, BALB/c 4.9, $U(6,6)=2$, $p=0.004$.

The acute effects of these doses of haloperidol on the activity of the cholinergic system in striatum and hippocampus in both strains were assessed by measures of the kinetics of the sodium dependent high affinity choline uptake mechanism in crude synaptosomal (P2) preparations. Preliminary studies, in agreement with those of Atweh *et al.* [3] showed that acute dopaminergic blockade leads to an activation (disinhibition) of cholinergic activity and results in an increased Vmax value for this mechanism with no change in apparent $K_T$ (Fig. 1). We, therefore, routinely assessed the increase in high affinity choline uptake velocity using a single concentration (0.25 $\mu$M) of methyl-$^{3}$H choline chloride. Groups of mice were sacrificed at 30 min and at 60 min following injection. It may be seen from Figs. 1 and 2 that control levels for sodium dependent high affinity choline uptake velocity show significant strain differences as previously reported [13] with C57BL/6 showing higher values in both brain regions (striatum: C57BL/6: 69.6±3.2; BALB/c: 50.1±2.4 pmoles/4 min/mg Pr±S.E.M. $p$ for strain difference $<$ 0.001; Hippocampus: C57BL/6: 21.6±0.5, BALB/c: 18.1±0.4 pmoles/4 min/mg Pr±S.E.M. $p$ for strain difference $<0.01$).

Haloperidol treatment produces significant activation of striatal high affinity choline uptake kinetics in C57BL/6 mice at each dose and time whereas in BALB/c no significant change in uptake velocity was noted at either time for 0.5 mg/kg and 1.0 mg/kg doses (Fig. 1a). Significant activation of striatal high affinity choline uptake velocity was observed in BALB/c following doses of 2 mg/kg at both times though the amplitude of this effect was much less (±18% at 60 min) than in C57BL/6 (±60% at 60 min). Acute treatment with haloperidol was also observed to result in the activation of high affinity choline uptake kinetics in hippocampus and indeed similar patterns with respect to dose, time and strain to those observed in striatum were noted. Thus, doses of 1 mg/kg and 2 mg/kg produce significant activation in C57BL/6 at both times whereas in BALB/c activation is only significant following 2 mg/kg doses (Fig. 1b). Once again the amplitude of the activation is much greater in C57BL/6 at this dose (+34% at 60 min) than in BALB/c (+14% at 60 min).

### *Strain Comparison of the Acute Effects of Sulpiride*

Groups of mice of both strains were treated by acute systemic injection with doses of 50 mg/kg, 100 mg/kg sulpiride or saline. In contrast to the effects observed following haloperidol, treatment with sulpiride was frequently observed to induce an initial transient (5 min) increase in locomotor activity in C57BL/6 mice which was followed by significant reduction in locomotor activity from 30 min. Some light limb tremor and occasional burrowing behaviour were observed in this strain but these effects did not appear to be dose-related. In BALB/c mice some reduction in locomotor activity scores was also noted following a latency of about 30 min though this was less marked than for C57BL/6. Cumulative mean activity ratings (arbitrary scale) for the second 30 min observation period were C57BL/6: 2.2, BALB/c 2.9, n.s. neither strain exhibited spontaneous or induced catalepsy at any time with either dose.

Cholinergic activity in striatum and hippocampus was assessed, as previously, using measures of the kinetics of high affinity choline uptake at 30 min and 60 min following treatment. As was the case for haloperidol acute treatment with sulpiride was observed to produce a dose-dependent increase in cholinergic activity in both brain regions and with a similar pattern of strain dependence. Thus in C57BL/6 striatum (Fig. 2a) significant activation of uptake was observed with each dose and time whereas in BALB/c significant activation was only observed (+9%, $p<0.02$) at 60 min following 100 mg/kg doses. Maximal activation observed in C57BL/6 striatum (100 mg/kg 60 min) was +18%. In hippocampus (Fig. 2b) a very similar pattern of activation of

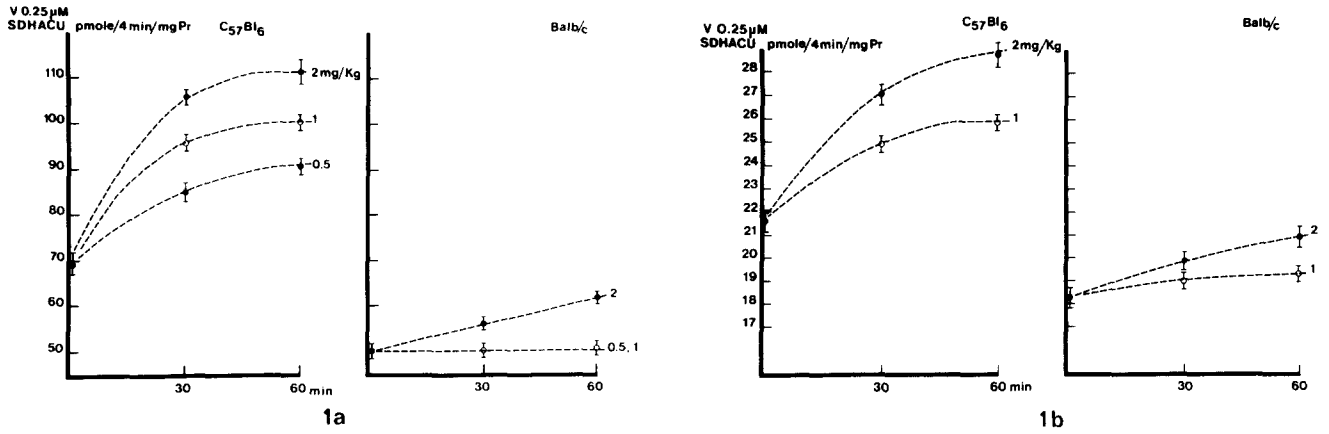


FIG. 1. Strain comparison of the acute effects of haloperidol on the velocity of high affinity choline uptake by crude synaptosomal preparations of striatum (Fig. 1a) and hippocampus (Fig. 1b). Haloperidol was injected systemically at doses indicated and high affinity choline uptake kinetics determined in groups of mice (n=3) at 30 min and 60 min following treatment and compared with saline injected control groups. Data have been pooled from control groups: striatum (n=18), hippocampus (n=12) and results plotted as pmoles of choline uptake/4 min/mg of protein ±S.D. Apparent $K_T$ ($\mu$M) of the sodium dependent high affinity choline uptake of striatal P2 fractions from vehicle injected C57BL/6 mice was 0.81±0.07 (n=3) and from mice injected with 2.0 mg/kg IP haloperidol (30 min) 0.86±0.09 (n=3) n.s.

choline uptake was observed with maximal activation in C57BL/6 (100 mg/kg 60 min) being +25%. In BALB/c doses of 50 mg/kg were without effect on high affinity choline uptake at either time whereas 100 mg/kg doses produced a 13% activation ($p<0.01$) at 60 min.

*Strain Comparison of the Sensitivity of the Striatal Dopamine Sensitive Adenylate Cyclase System*

Comparative studies on the dopamine sensitivity of the adenylate cyclase of striatal tissue from C57BL/6 and BALB/c mice were conducted using EGTA-washed particulate fractions as described in the Method section since we found that dopamine stimulation of this enzyme using homogenates was relatively low (less than 50% increase over basal activity) and showed rather variable responses to GTP addition. In each experiment we compared basal cAMP production, activity in the presence of GTP ($10^{-6}$ M to $10^{-4}$ M) and activity in the presence of both GTP and dopamine ($10^{-6}$ M to $10^{-4}$ M). It may be seen from Table 1 that no significant strain differences were observed either for basal cAMP production or for activity in the presence of an optimal concentration of GTP ($10^{-5}$ M); concentrations in excess of $10^{-5}$ M being observed, in agreement with previous studies [9,18] to produce inhibition of adenylate cyclase activity. Further we were unable to demonstrate any significant strain differences either for maximal stimulation of adenylate cyclase activity by dopamine ($10^{-4}$ M) or for the concentration of dopamine ($K_a$) which produced half-maximal activation. Comparative studies on the *in vitro* sensitivity of the dopamine stimulated adenylate cyclase to inhibition by haloperidol at doses ranging from $10^{-8}$–$10^{-5}$ M also provided no evidence for significant strain differences in $K_i$.

*Strain Comparison of the Acute Effects of Apomorphine*

Groups of mice from both strains were treated by acute systemic injection of apomorphine hydrochloride in doses of 0.5 and 5 mg/kg or physiological saline. The lower dose of 0.5 mg/kg was observed to produce, following a latency of approximately 5 min, an initial effect on posture in both strains affecting the splay of the rear limb position. This effect on posture induced a waddling type locomotion with some consequent reduction in locomotor activity in both strains. Mice of the BALB/c strain were also observed to exhibit occasional sniffing behaviour. Higher doses of 5 mg/kg produced highly significant strain differences in stereotypy. Although initial effects were observed in both strains, as for 0.5 mg/kg doses, on rear limb position only moderate reductions in locomotor activity and intermittent sniffing and gnawing behaviours were observed in C57BL/6 mice whereas mice of the BALB/c strain continuously exhibited elevated stereotypy scores over the entire observation period. Cumulative mean stereotypy ratings for the second 30 min period of observation were C57BL/6 1.6, BALB/c 5.7; $U(6,6)=0, p=0.001$. All treated BALB/c mice showed constant sniffing behaviour, curved back; tail elevation, almost total eyelid closure and rearing. This latter behaviour was initially observed during the first 15 min as a slow and rhythmic raising of the head to a vertical position, then evolving into a totally upright body posture frequently maintained for periods in excess of 15 seconds and often without support of the sidewalls of the cage [30].

Cholinergic activity in hippocampus and striatum of treated and control mice was evaluated using measures of high affinity choline uptake kinetics at 30 min following treatment during the peak of the behavioural effects. No significant changes in high affinity choline uptake kinetics were observed in either brain region of either strain for any dose. Slight inhibition of uptake velocity was observed in both hippocampus and striatum of BALB/c and in C57BL/6 hippocampus at the higher dose though these effects were never greater than 10% and did not achieve statistical significance.

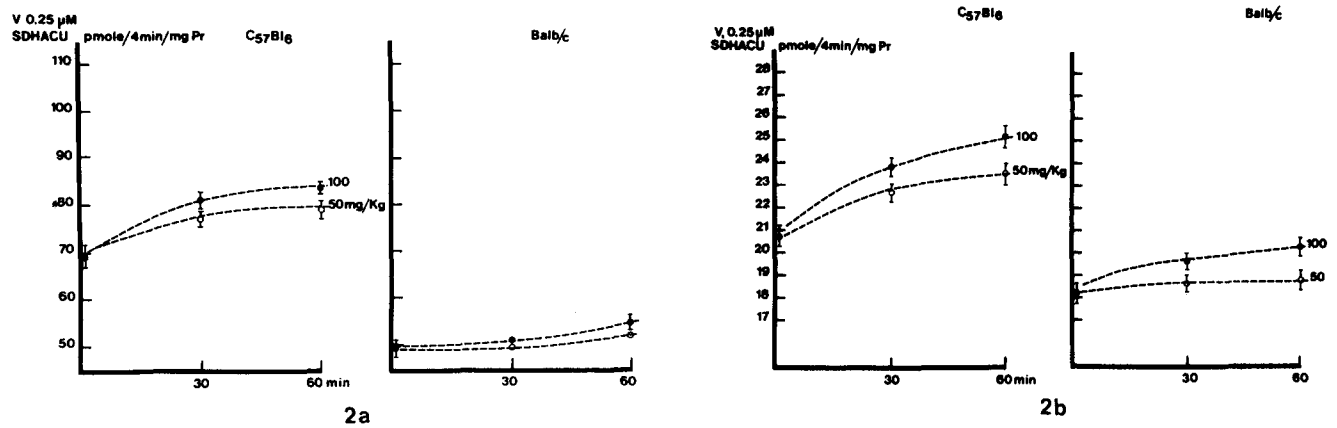


FIG. 2. Strain comparison of the acute effects of sulpiride on the velocity of high affinity choline uptake by crude synaptosomal preparations of striatum (Fig. 2a) and hippocampus (Fig. 2b). Sulpiride was injected systemically at doses indicated and high affinity choline uptake kinetics determined in groups of mice (n=3) at 30 min and 60 min following treatment and compared with saline control groups. Data have been pooled from control groups (n=12) and results plotted as pmoles choline uptake/4 min/mg of protein ±S.D.

## DISCUSSION

In the present investigation we have attempted to probe the functional consequences for dopaminergic-cholinergic interactions in hippocampus and striatum of genotypic variation in dopaminergic neurotransmission. Acute blockade *in vivo* of dopamine receptors using haloperidol results in a monophasic activation of both hippocampal and striatal cholinergic activity consistent with a dis-inhibition mechanism in both brain regions. Highly significant genotypic variation in neuroleptic sensitivity was observed by both behavioural and biochemical criteria with C57BL/6 mice exhibiting significantly higher catalepsy scores and greater amplitude of cholinergic activation as a function of dose in both regions than BALB/c mice.

These experiments using increasing doses of haloperidol demonstrate that the amplitude of the resulting dis-inhibition of cholinergic activity in both striatum and hippocampus shows marked strain dependence. These observations may be interpreted as being in agreement with the reports [6,39] that the density of striatal dopamine receptor sites shows genotypic variation. Further this is reflected functionally as mediating different levels of tonic inhibition of cholinergic activity.

Dopamine receptors have been divided into two major categories on the basis of their association with adenylate cyclase (type D1) or their independence (type D2) on this enzyme [25,41]. In a parallel series of experiments using acute *in vivo* treatments of mice with the reportedly specific D2 antagonist sulpiride [24, 42, 45, 47] we attempted to test the hypothesis that the dopaminergic control of cholinergic activity in these two brain regions was being mediated via D2 type receptors. Acute treatment with sulpiride also results in a similar increase in high affinity choline uptake kinetics in both striatum and hippocampus implying that the tonic inhibition of cholinergic activity in both brain regions is mediated via D2 receptors. This conclusion is in agreement with those of Sethy [37,38] and Scatton [35,36]. As was the case with haloperidol, the amplitude of activation as a function of dose was strain-dependent with C57BL/6 again showing greater sensitivity than BALB/c mice. This may be interpreted as indicating that the genotypic variation in the density of striatal ($^3$H) spiroperidol and ($^3$H) ADTN binding

TABLE 1

STRAIN COMPARISON OF DOPAMINE STIMULATED ADENYLATE CYCLASE ACTIVITY IN EGTA-WASHED STRIATAL PARTICULATE FRACTIONS

| | Adenylate cyclase activity pmoles cAMP/mg Protein/min ± S.D. (n=6) | | |
|---|---|---|---|
| | C57BL/6 | BALB/c | *p* |
| Basal | 116 ± 10 | 122 ± 8 | n-s |
| Basal + GTP $10^{-5}$ M | 198 ± 12 | 192 ± 14 | n-s |
| Basal + GTP $10^{-5}$ M + Dopamine $10^{-4}$ M | 352 ± 24 | 336 ± 20 | n-s |
| $K_a$ Dopamine μM | 11.1 ± 0.8 | 9 ± 0.6 | n-s |
| $K_i$ Haloperidol μM | 0.47 ± 0.04 | 0.5 ± 0.03 | n-s |

sites of approximately twofold observed by Severson *et al.* [39] for the BALB/c and C57BL/6 strains involves the D2 receptor population. However much higher doses of sulpiride were needed to produce activation of cholinergic activity than were required using haloperidol and the amplitude of activation produced was not as great. These variations in potency have been interpreted on the basis of the poor brain penetration and the low lipid solubility of sulpiride [20, 23, 47]. It is, therefore, of interest to note that although maximal amplitudes of activation were not as great, in contrast to haloperidol, the amplitude of the activation of cholinergic activity in the hippocampus was equivalent to or even slightly greater than that observed in striatum. This regional selectivity of the action of sulpiride in brain has been observed by others in the rat [28,45] and may be attributable to regional variation in the relative density of the D2 receptor sub-population. It is also noteworthy that acute dopaminergic blockade *in vivo* results in activation of cholinergic activity with highly similar temporal kinetics in both striatum and hippocampus. Whereas in striatum the A9 dopamine terminals have been considered to synapse directly with cholinergic interneurones [17,37], the A10 dopamine system appears to terminate in the lateral septum and exerts a modulatory influence on septohippocampal cholinergic activity via a more indirect mechanism involving γ-amino-butyrate (GABA) interneurones [32]. Strain differences in the D1 receptor population are not excluded, however, and indeed previous studies [11, 31, 44] have produced evidence for genotypic variation in the sensitivity of the dopamine stimulated adenylate cyclase system from mouse striatal homogenates. We, thus, decided to determine whether evidence for such possible genotypic variation in the sensitivity of the striatal dopamine sensitive adenylate cyclase system could be observed between C57BL/6 and BALB/c strains and, thus, contribute to the observed genotypic variation in the inhibitory dopaminergic control of cholinergic activity. Comparative *in vitro* studies using striatal membranes from both strains provided no evidence for significant genotype dependent differences in kinetic aspects of the D1 dopamine sensitive adenylate cyclase complex. Neither the slope of the curve for the activation of adenylate cyclase by dopamine nor that for the inhibition of dopamine stimulation by haloperidol was observed to yield evidence for a possible twofold variation in D1 receptor density. However, since fractional receptor occupancy may produce a maximum physiological response of the cyclase system such variations in receptor density are not excluded and must await direct quantitation by the use of specific radioligands.

We were interested by the possibility that if these strain dependent effects of dopaminergic antagonists were being mediated by genotypic variation in receptor density than symmetrically opposite behavioural and neurochemical strain sensitivity to agonists might be observable [15,19]. To probe this eventuality we used acute treatments with apomorphine, since this has been considered as a convenient means to assess the stimulation of striatal dopamine receptors in mice [30]. The behavioural results were in agreement with our postulate since BALB/c mice showed significantly greater stereotypy and rearing scores at each dose than did C57BL/6 mice though this was most evident at 5.0 mg/kg doses. Severson *et al.* [39] did not observe significant differences in stereotypy scores between C57BL/6 and BALB/c using peritoneal injection of 1.0 mg/kg apomorphine but did observe similar qualitative and quantitative strain differences, as we report for C57BL/6, with the CBA/J strain. The CBA/J strain was also reported by these authors to exhibit greater sensitivity to the cataleptogenic action of 0.6 mg/kg haloperidol at periods of between 2 and 6 hours following injection than either the C57BL/6 or BALB/c strains. An opposite pattern of strain sensitivity to the cataleptic effects of haloperidol and other neuroleptics has, however, been reported by Fink *et al.* [16] for the CBA/J and BALB/c strains using much higher doses of haloperidol (4 and 8 mg/kg). Interestingly Severson *et al.* [39] report that the C57BL/6 and CBA/J strains both exhibit 40–50% lower striatal dopamine receptor densities, as assessed by spiroperidol and ADTN binding compared to the BALB/c strain. In contrast to these strain differences in sensitivity to the behavioral effects of apormorphine we were unable to demonstrate any significant effect in either strain for the postulated inhibition of the rate of high affinity choline uptake either in striatum or hippocampus. While this observation is in agreement with the negative result of Atweh *et al.* [3] for 3.0 mg/kg apomorphine in rat striatum, several authors noted that similar doses induce the expected increase in steady state acetylcholine concentration and/or decrease in turnover rate [7, 17, 35, 37, 43]. This lack of effect of apomorphine on striatal high affinity choline uptake kinetics, similarly to the negative effect of pentobarbital, constitute discrepancies for the proposal [23] that intra-cellular acetylcholine levels control the uptake mechanism and have lead Murrin *et al.* [29] to state that there appears to be no simple relationship between acetylcholine levels and choline uptake in all cases. We are currently attempting to resolve this problem of demonstrating genotypic variation in the inhibition of striatal and hippocampal high affinity choline uptake using specific D2 agonists.

In conclusion, acute treatments with dopaminergic antagonists of differing receptor sub-type selectivities have revealed evidence for significant genotypic variation in dopaminergic-cholinergic interactions in mice. Strain differences in behavioural sensitivity and in the degree of tonic inhibitory control of striatal and septal-hippocampal cholinergic activity constitute further evidence for genotypic variation in dopamine receptor density and function in these brain regions and particularly implicate the D2 receptor sub-population. These strain-dependent variations in the density of the striatal dopaminergic neurotransmission system have recently been observed [5] to occur post-natally and have been interpreted as representing genetic influences on the post-natal survival of dopamine neurones. Genotypic variation in the expression of certain behaviours (e.g., learning and long term memory capacity [21,22]) may, therefore, be linked via secondary influences on the control of central cholinergic activity to developmental mechanisms governing dopamine cell survival.

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