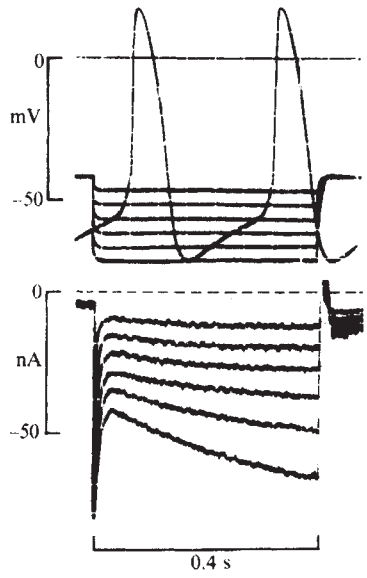

Fig. 3 *a*, Superimposition of pacemaker activity and voltage-clamp hyperpolarisations from a holding potential of −42 mV in the same preparation; *b*, currents recorded during the clamp hyperpolarisations. Note that the time-dependent changes in i_f are greater at more hyperpolarised levels.

With this method it can be shown that in the rabbit SA node adrenaline does indeed increase both slow inward current ($i_{Ca/Na}$) and outward potassium current (the current termed i_K by Difrancesco Noma and Trautwein[10]). Figure 2 shows the increase in peak $i_{Ca/Na}$ current caused by adrenaline during a 10-mV depolarising voltage-clamp pulse and the increase in the i_K-current tail recorded on return to the holding potential of −45 mV. These results correlate with previous findings that adrenaline increases both the slow inward and the plateau outward current in other cardiac tissues (for review see ref. 11).

As indicated in Fig. 1, the positive chronotropic effect of adrenaline is mediated by an increased rate of pacemaker depolarisation (PD). An increase of i_K will, however, tend to decelerate the PD. Although the larger $i_{Ca/Na}$ would by itself accelerate the PD in its later stages immediately preceding the upstroke, is it unlikely that combined with the augmented i_K it could account for the increased rate of change of voltage during the whole course of the PD seen in adrenaline.

What, then, is the mechanism of the increased pacemaker rate seen in adrenaline? We have found that an additional current, which has been previously noted in the rabbit SA node[9] and in the frog sinus venosus[12] and which we will call i_f, is important both in normal pacemaker activity and in adrenaline-induced acceleration. Hyperpolarising voltage-clamp pulses from a holding potential of −42 mV (Fig. 3) reveal that i_f is a slowly increasing inward (or decreasing outward) current change whose amplitude increases with the clamp pulse amplitude. In Fig. 3 the voltage protocol has been superimposed on a record of

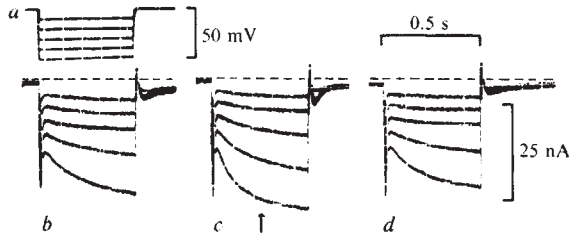

Fig. 4 Increase of i_f in adrenaline. i_f changes are activated by voltage-clamp hyperpolarisations from a holding potential of −36 mV. *a*, Voltage protocol used throughout the experiment; *b*, control; *c*, during adrenaline, 10^{-7} M; *d*, return.

spontaneous activity in the same preparation to show the relevance of i_f to the potential range of the pacemaker depolarisation. Addition of adrenaline, 10^{-7} M, causes a substantial and reversible increase in the time-dependent change of i_f, as shown in Fig. 4. In the unclamped preparation such an increase would cause a faster PD, as observed during adrenaline action in Fig. 1.

Investigating the ionic nature of the i_f current system is difficult as it is not possible to hyperpolarise the small preparations used by more than 50 mV without risking membrane injury and consequent breakdown of the voltage clamp. We have therefore been unable to determine whether i_f has a reversal potential close to the equilibrium potential for K^+ ions, that is, at about −100 mV, which would show whether or not it could be equated with the i_{K_2} current of the Purkinje fibre. Nevertheless, the resemblance to i_{K_2} is striking: i_{K_2} is deactivated by hyperpolarisations into the same voltage range as i_f and the change in i_f produced by adrenaline strongly resembles that produced in i_{K_2} by a voltage shift of its kinetics. Regardless of whether it becomes possible to test this resemblance further experimentally, it is already clear that i_f has an important role both in normal rhythmic activity in the SA node and in mediating the response to adrenaline.

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Role of the dendritic release of dopamine in the reciprocal control of the two nigro-striatal dopaminergic pathways

USING cats implanted with push–pull cannulae, we have previously demonstrated a reciprocal regulation of the activity of the two nigro-striatal dopaminergic pathways. Asymmetric fluctuations in the spontaneous release of dopamine(DA) were simultaneously seen in the two caudate nuclei (CN)[1]. As revealed by the changes in DA release from nerve terminals, the unilateral nigral application of DA[1] or of drugs enhancing the dendritic release of DA, such as amphetamine or benztropine (2), reduced the activity of ipsilateral dopaminergic neurones and induced an opposite effect in the contralateral side. Conversely, the unilateral nigral blockade of dopaminergic transmission by local application of neuroleptics enhanced the release of DA in the ipsilateral CN and decreased the transmitter release in the contralateral structure[2]. Opposite changes in the release of DA were also seen in the two CN under unilateral delivery of sensory stimuli[3] or during unilateral electrical stimulation of the cerebellar dentate nucleus[4]. These latter effects were associated with asymmetric changes in the dendritic release of DA in the two substantia nigrae (SN) which were in an opposite direction to those seen in the two CN (an increased

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release of DA from nerve terminals corresponded to a decreased release of the transmitter from dendrites and vice versa). These experiments indicated that DA which is released from dendrites in one SN regulates the activity of the ipsilateral dopaminergic neurones; they also suggested that it contributes to the control of the contralateral dopaminergic neurones by influencing the dendritic release of DA in the contralateral SN. This hypothesis was confirmed in the present study by measuring the changes in DA release from nerve terminals and dendrites of the two pathways under pharmacological blockade or facilitation of dopaminergic transmission in one SN.

Adult cats of both sexes were anaesthetised with halothane and implanted with four push–pull cannulae (0.9 mm diameter), one in each SN and each CN, to measure continuously the release of ^{3}H-DA synthesised from ^{3}H-tyrosine, as previously described[5]. An artificial cerebrospinal fluid (CSF) containing ^{3}H-tyrosine (50 Ci mmole^{-1}, 50 μCi ml^{-1}) was introduced in each structure at a rate of 1 ml h^{-1} and ^{3}H-DA was estimated in serial 10-min superfusate fractions. ^{3}H-DA was separated from ^{3}H-tyrosine and ^{3}H-labelled metabolites by ion-exchange chromatography and alumina adsorption. α-Methyl-*p*-tyrosine (α-MT, 10^{-4}M), an inhibitor of DA synthesis, or (+)amphetamine (10^{-6} M) were applied to the left SN 22 h after the onset of superfusion of the four structures with ^{3}H-tyrosine to block or facilitate local dopaminergic transmission, respectively.

The unilateral nigral application of α-MT for 60 min markedly stimulated the release of ^{3}H-DA in the ipsilateral CN and induced an opposite effect in the contralateral structure. Moreover, in contrast to that observed in the ipsilateral side, this treatment stimulated the dendritic release of ^{3}H-DA in the contralateral SN (Fig. 1). The unilateral nigral application of (+)amphetamine for 10 min not only induced asymmetric changes in the release of ^{3}H-DA in both CN (as previously shown[2]), but also in both SN. These effects were in opposite directions to those induced by αMT (Fig. 2).

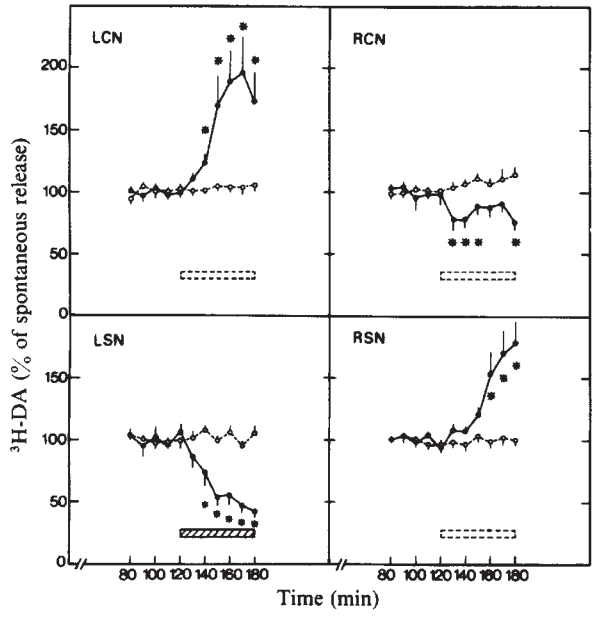

Fig. 1 Effects of α-methyl-*p*-tyrosine application into the left substantia nigra on the release of ^{3}H-dopamine from the two caudate nuclei and the two substantia nigrae. Four push–pull cannulae were simultaneously implanted in the left (LCN) and the right (RCN) caudate nuclei and in the left (LSN) and the right (RSN) substantia nigrae in anaesthetised cats. The four structures were perfused with an artificial CSF containing L[3,5-^{3}H]tyrosine (50 Ci mmol^{-1}, 50 μCi ml^{-1}, 1 ml h^{-1}. ^{3}H-Dopamine (^{3}H-DA) was estimated in 10-min successive superfusate fractions. The average quantities of ^{3}H-DA released in superfusate fractions of the SN (0.4 nCi) and the CN (0.6 nCi) were, respectively, 20 and 30 times the blank value. α-Methyl-*p*-tyrosine (α-MT, 10^{-4} M) was introduced for 60 min into the CSF superfusing the LSN (hatched bar). In each animal and for each cannula, ^{3}H-DA in each successive fraction was expressed as a percentage of an average spontaneous release calculated from the five fractions collected before the treatment. Data are the mean ±s.e.m. of results obtained with five animals (—•—). $*P < 0.05$ when compared with corresponding control values obtained in five untreated animals (---○---). ▨, α-MT application.

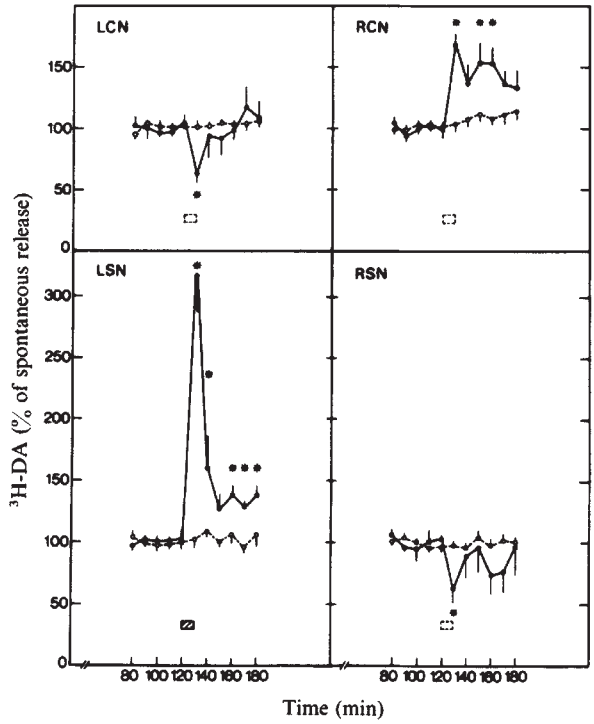

Fig. 2 Effects of (+)amphetamine application into the left substantia nigra on the release of ^{3}H-dopamine from the two caudate nuclei and the two substantia nigrae. Five animals were treated as described in Fig. 1 except that (+)amphetamine (10^{-6} M) was applied for 10 min into the left substantia nigra (LSN). Data are calculated and expressed as in Fig. 1. $*P < 0.05$ when compared with corresponding control values obtained in five untreated cats (---○---). ▨, (+)Amphetamine application.

To confirm that the contralateral effects induced by the unilateral application of α-MT or (+)amphetamine were mediated by the changes in DA release induced in the ipsilateral SN and were not secondary to those observed in the ipsilateral CN, these drugs were applied to the left CN and ^{3}H-DA was simultaneously measured in both CN and both SN. α-MT (10^{-4}M) and (+)amphetamine(10^{-6}M) which, respectively, reduced and enhanced the local release of ^{3}H-DA in the left CN, failed to change ^{3}H-DA release in the contralateral CN or SN (Figs. 3, 4). Also, marked changes in the dendritic release of ^{3}H-DA were seen in the ipsilateral SN (Figs. 3, 4). They were parallel to those detected in the CN, as the dendritic release of ^{3}H-DA was reduced by α-MT and enhanced by (+)amphetamine.

Several conclusions on the influences of DA can be drawn from the present study and are summarised in Fig. 5. First, the activation of ipsilateral dopaminergic neurones induced by the unilateral application of α-MT demonstrates that DA released from dendrites tonically inhibits the activity of the ipsilateral neurones. This may mainly result from a reduced availability of DA at dopaminergic receptor sites located either on dopaminergic cells or their dendrites[6,7] or on nigral afferent fibres[8,9] (Fig. 5*a*). Second, the effects of α-MT and (+)-amphetamine on the dendritic release of ^{3}H-DA imply that these drugs, respectively, stimulated or inhibited the firing rate of the ipsilateral dopaminergic neurones through their effects on

the dendritic release of DA. Thus, the changes in DA dendritic release induced by local modifications of the dopaminergic transmission in the ipsilateral CN further support the postulated[10] existence of a striato-nigral neuronal loop controlling the activity of the dopaminergic neurones (Fig. 5*b*). Third, the asymmetric changes in DA release seen in both CN under the unilateral application of various stimuli[1–4] cannot be related to modifications of ^{3}H-DA release in one of the CN (Fig. 5*c*). Indeed, the changes in ^{3}H-DA release from nerve terminals in one CN induced by the local application of α-MT or (+)-amphetamine did not affect ^{3}H-DA release in the contralateral CN (Figs 3, 4). We therefore postulate that the asymmetric changes in DA release observed in the two CN result from the change in the dendritic release of DA occurring in one SN (Fig. 5*d* or *e*). This is well illustrated by the experiments shown in Figs 1 and 2. DA released from dendrites in one SN must thus

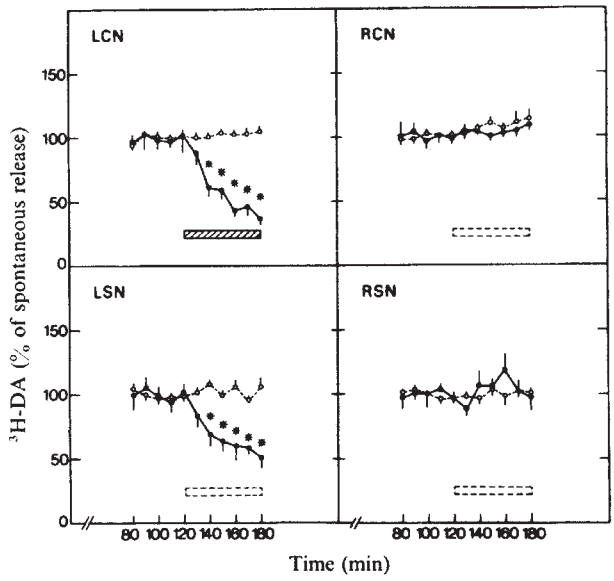

Fig. 3 Effects of α-methyl-*p*-tyrosine application into the left caudate nucleus on the release of ^{3}H-dopamine from the two caudate nuclei and the two substantia nigrae. Five animals were treated as described in Fig. 1 except that α-methyl-*p*-tyrosine (α-MT, 10^{-4} M) was applied for 60 min into the left caudate nucleus (LCN). Data are calculated and expressed as in Fig. 1. $*P < 0.05$ when compared with corresponding control values obtained in five untreated cats (---○---). ▨, α-MT application.

regulate the activity of a nigral efferent pathway involved in the control of the activity of the contralateral dopaminergic neurones. This could occur as DA acts not only on the dopaminergic neurones in the pars compacta but also on other neurones in the pars reticulata[11]. DA could also indirectly affect the activity of the pars reticulata neurones by modifying the release of transmitters from nigral afferent fibres[12]. As cells of the pars reticulata project to several structures in the brain[13–17], the pathway involved remains to be determined. However, we can still discuss whether the final input of this pathway occurs in the contralateral SN (Fig. 5*e*) or contralateral CN (Fig. 5*d*). Fourth, the effects of unilateral nigral αMT or (+)amphetamine indicate that the asymmetric changes in the dendritic release of DA in both SN are responsible for the opposite asymmetric changes in DA release in both CN. Indeed, the changes in the dendritic release observed in the right SN during the application of these drugs in the left SN cannot be related to the opposite changes in ^{3}H-DA release from nerve terminals seen in the contralateral CN (Fig. 5*d*). If this was the case, parallel changes should occur in the CN and the SN as observed during the local application of (+)amphetamine or α-MT in the CN (Figs 3, 4). As there is apparently no direct anatomical connection between

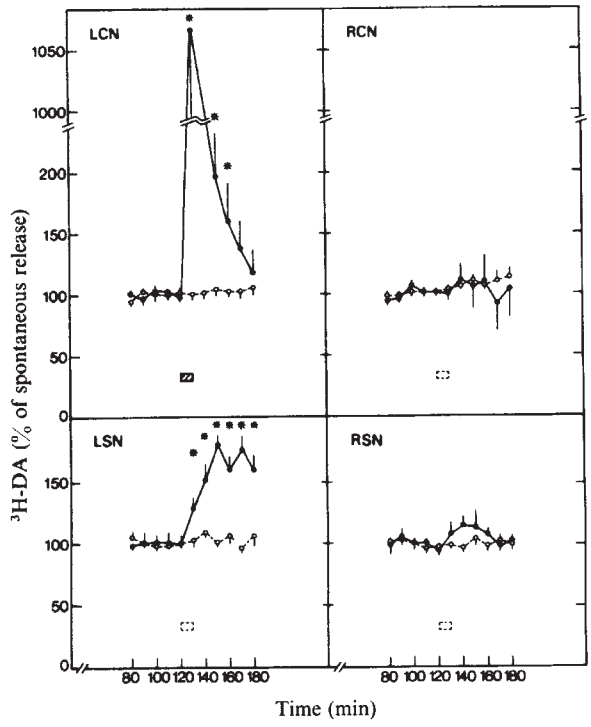

Fig. 4 Effects of (+)amphetamine application into the left caudate nucleus on the release of ^{3}H-dopamine from the two caudate nuclei and the two substantia nigrae. Seven animals were treated as described in Fig. 1 except that (+)amphetamine (10^{-6} M) was applied for 10 min into the left caudate nucleus (LCN). Data are calculated and expressed as in Fig. 1. $*P < 0.05$ when compared with corresponding control values obtained in five untreated cats (---○---). ▨, (+)Amphetamine application.

the two SN, a polysynaptic pathway activated or inhibited by DA released from dendrites in one SN must be involved in the reciprocal control of the dendritic release of DA in the contralateral SN and thus in the regulation of the activity of the contralateral dopaminergic neurones (Fig. 5*e*).

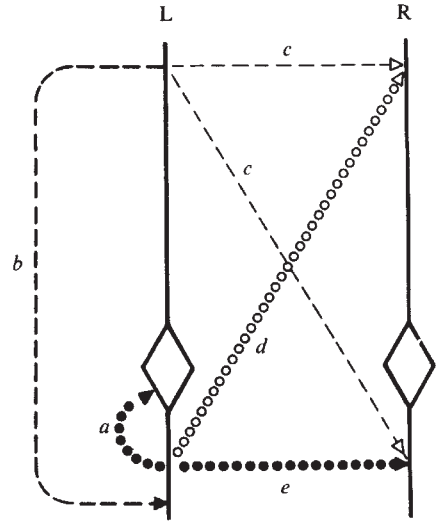

Fig. 5 Influences of dopamine released from dendrites and nerve terminals. Possible direct and indirect influences of dopamine (DA) released from nerve terminals (– – –, – – –) or dendrites (○, ●) of the left (L) dopaminergic neurones on both nigro-striatal dopaminergic pathways (L, R). Our results indicate the existence of the influences *a*, *b* and *e*, and exclude *c* and *d* (see text).

Finally, we should consider why no contralateral effects were observed during the local application of α-MT or (+)amphetamine in the left CN despite the changes in the dendritic release of ^{3}H-DA induced in the ipsilateral SN. Recent experiments suggest that the striato-nigral substance P neurones regulate the activity of the dopaminergic neurones by acting on DA dendritic release[18]. Moreover, in these experiments, there was no effect in the contralateral SN and CN[19], as was the case when dopaminergic drugs were applied in the left CN (Figs. 3, 4). Therefore, the striato-nigral substance P neurones may not only regulate the activity of the ipsilateral nigro-striatal dopaminergic neurones but could also influence the nigral cells involved in the reciprocal regulation of the two dopaminergic systems, and thus counteract the effect of DA on these cells. In fact, substance P nerve terminals are distributed in both the pars compacta and the pars reticulata[20–22] and substance P activates most cells in the SN[23,24]. Furthermore, in contrast to that observed with substance P and its antibody, the unilateral nigral application of agonists or antagonist of γ-aminobutyric acid affected the release of DA in the contralateral CN[25]. These effects were distinct from those induced by dopaminergic drugs, as in all cases the changes in ^{3}H-DA release seen in the two CN were symmetric.

Regarding their role in the coordination of sensory motor processes, it has previously been assumed that the nigro-striatal dopaminergic neurones act merely by modulating the activity of striatal cells. The demonstration of the dendritic release of DA, its important fluctuations in various physiological and pharmacological situations, and its role in regulating the activity, not only of the dopaminergic neurones but also of other nigral efferent pathways, indicate that the nigro-striatal dopaminergic neurones also control the delivery of messages originating from the SN. Such processes, which could be shared by other neurones, will undoubtedly influence our view of the transfer of information in the central nervous system.

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The role of mitogenic lectins in T-cell triggering

THE process of T-lymphocyte activation requires the participation of metabolically active non-θ-bearing accessory cells[1]. As first conclusively shown by Habu and Raff[2], this requirement is also true of lectin-dependent triggering. Thus, T-cell activation by mitogenic lectins depends on the presence of Ia-positive non-T cells[3,4]. The mere binding of lectins such as concanavalin A (Con A) to the surface membrane of T cells does not trigger the cells to go through the mitotic cycle. This can only be achieved by the activity of growth factors present in conditioned media (CM) from Con A-stimulated cell cultures[5]. Such growth factors, however, cannot activate normal, resting T cells, although they are competent to maintain activated T cells in exponential growth for indefinite periods of time[6]. Hence, to be mitogenic a lectin must not only induce the *in situ* production of T-cell growth factors, but must also render resting T cells sensitive to the mitogenic activity of these growth factors. In this report we demonstrate that the binding of Con A to purified T cells can in a relatively short time, modify their functional sensitivity to growth factors, even though this binding is not sufficient to stimulate them to proliferate.

In our initial experiments we have used CM obtained at 24 h from Con A-stimulated spleen cell cultures. CM were always tested for T-cell growth factor activity on cultures of purified T-cell blasts[4]. Normal spleen cells, when cultured in such CM where the lectin has been inactivated (in our experiments by supplementation with the specific inhibitor sugar α-methyl-D-mannoside (α-MM)) are not induced to grow[6] (Figs 1, 2 and Table 1). To demonstrate that direct interaction with mitogenic lectins renders T cells sensitive to growth factors, we incubated normal spleen cells in medium, either alone or with 5 μg ml^{-1} Con A, for various periods of time. The cells were then washed

Table 1 Direct interaction of Con A with T cells induces responsiveness to growth factors by expression of acceptor sites

Expts 1 and 2

Additions to cultures	Spleen cell responses (c.p.m. per culture × 10^{-3}) after: Anti-Ig column Expt 1	Anti-Ig column Expt 2	Anti-I-A treatment and anti-Ig column Expt 1	Anti-I-A treatment and anti-Ig column Expt 2
None	1.6±0.2	1.5±0.2	0.3±0.2	0.4±0.3
Con A	339.3±11.2	165.2±4.9	3.6±0.4	2.6±0.6
CM	ND	3.7±0.3	2.1±0.4	0.8±0.3
Con A+CM	ND	81.4±5.7	79.4±3.9	45.9±3.9

Expts 3 and 4

		T-cell blast response (c.p.m. per culture × 10^{-3}) to CM absorbed on: Expt 3	Expt 4
None	—	1.8±0.4	0.6±0.4
Con A	—	1.3±0.2	1.3±0.4
CM	—	25.8±2.7	18.6±1.9
CM	Normal spleen cells	19.3±2.5	15.4±2.1
CM	4-h Con A spleen cells	12.9±1.6	7.1±0.9
CM	T-cell blasts	9.2±2.0	0.7±0.2

In expts 1 and 2 normal C3H/HeJ spleen cells either untreated or incubated for 30 min in the cold with monoclonal anti-I-A (clone H-116/32, see ref. 14) antibodies were passed over anti-Ig columns (13) and then stimulated in culture (2×10^5 ml^{-1}) with Con A (5 μg ml^{-1}) or a standard preparation of CM at a 50% (v/v) final concentration. ^{3}H-Thymidine uptake was measured on day 3 of culture. This type of experiment has been reproduced on six independent occasions. In expts 3 and 4 T-cell blasts kept in standard CM for over 2 weeks were cultured (5×10^4 ml^{-1}) in the presence of either Con A (5 μg ml^{-1}) or CM (50%, v/v, final concentration) which had been absorbed for 30 min in the cold with the indicated cells (2×10^8 ml^{-1} CM). Cells which had been exposed to Con A for 4 h were washed five times with 20 mg ml^{-1} α-MM before use in the absorption. T-cell blast responses were measured on day 2 after initiation of culture. Mixing experiments showing that the loss of activity in absorbed CM is not due to inhibitory activities have been carried out and partially presented elsewhere [6]. All results are shown as the mean values and s.d. from triplicate cultures. ND, not determined.

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