S14.5 The effects of 9-tetrahydrocannabinol on sympathetic cotransmission in the mouse vas deferens
Kennard, JAG; Sachdev, AR; Young, JS; Brain, Keith

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Interestingly, 20 min (noradrenergic and purinergic neurogenic contractions (10 pulses, 10 Hz, electrical field stimulation (EFS) provided by two platinum ring electrodes. The noradrenergic and purinergic components of contraction studies, vasa were suspended in 5 mL organ baths with drugs were added by pipette into the organ bath."

In conclusion, alpha-1-subtypes do not interact but provide independent dominance in the number of each subtype present, irrespective of which was dominant in functional terms.

'Pure' alpha-1-AR-subtype pharmacology found in the double knockouts provides quantitative standards. Alpha-1A-AR and alpha-1D-AR produced additive responses, each dominating in different vessels, and did not significantly compensate in knockout mice. Alpha-1D-AR contributes to sensitivity to phenylephrine even in resistance arteries. There was no correlation between the presence of a given subtype as shown by fluorescent ligand binding sites and its contribution to vascular contraction: this suggests that the signalling process is more critical than the quantitative presence of the receptor. Similar distribution of fluorescent binding in the double KOs and WT indicates that in native SMC alpha-1-AR-subtypes do not influence each other's location. In conclusion, alpha-1-subtypes do not interact but provide independent alternative signals for autonomic regulation of the vasculature.

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S14.5 The effects of Δ9-tetrahydrocannabinol on sympathetic cotransmission in the mouse vas deferens
J.A.G. Kennard, A.R. Sachdev, J.S. Young, K.L. Brain (Department of Pharmacology, University of Oxford)

The mouse vas deferens is a well established model system for studying the effects of cannabinoids on sympathetic neuroeffector junctions [1]. This tissue is also frequently used to study cotransmission, studying the effects of cannabinoids on sympathetic neuroeffector cotransmission in the mouse vas deferens S14.5 The effects of cannabinoids on the release and downstream actions of each of these neurotransmitters when pharmacologically isolated has not been carried out in the vas deferens. Therefore, we conducted experiments using Δ9-tetrahydrocannabinol (ΔTHC) to investigate this.

Vasa deferentia were excised from male Balb/C mice (7–10 weeks) for use in contraction and confocal microscopy experiments. For contraction studies, vasa were suspended in 5 mL organ baths with electrical field stimulation (EFS) provided by two platinum ring electrodes. The noradrenergic and purinergic components of contraction were isolated using αβ-methylene ATP (1 μM) and prazosin (100 nM) respectively; drugs were added by pipette into the organ bath. ΔTHC (100 nM) caused a reduction in the peak amplitude of both noradrenergic and purinergic neurogenic contractions (10 pulses, 10 Hz, 0.5 ms width, supramaximal voltage) with a significant effect from 20 min (P<0.01, n = 4–5) and reaching a maximal effect by 30–40 min. Interestingly, ΔTHC caused a greater reduction in the amplitude of noradrenergic than purinergic contractions: at 40 min, noradrenergic contractions showed an average 72±14% reduction compared to vehicle controls whereas purinergic contractions were reduced by 15±14% only (P=0.01). In agreement with previous studies, the effect of ΔTHC upon neurogenic contractions was concentration-dependent and did not have a significant effect upon contractions caused by the application of the exogneous agonists phenylephrine (0.1–30 μM) and αβ-methylene ATP (10 nM–1 μM). For experiments using a range of EFS train lengths (1–200 pulses), ΔTHC (100 nM) significantly increased the peak area for noradrenergic contractions at 200 pulses (P<0.05, n = 6) with an average 134±37% increase in peak area compared to vehicle controls. No significant effect was observed upon purinergic contractions.

For imaging experiments, vasa were orthogradely filled with the Ca2+ indicator Oregon Green 488 BAPTA-1 10 kDa dextran to identify sympathetic terminals on the stage of a Leica NT confocal microscope [2]. Upon imaging electrically-induced Ca2+ transients in NTVs, ΔTHC (100 nM) reduced the relative change in ΔF/F0 observed after a single field stimulus, compared to vehicle controls (P<0.0005). On average, ΔTHC resulted in a 20±3% reduction in the relative change of ΔF/F0 (P=0.0001), whereas vehicle produced no significant change 1±3% increase (P>0.05).

These results show that ΔTHC reduces the release of NA and ATP from sympathetic nerves in the mouse vas deferens, possibly by reducing Ca2+ influx into NTVs. The effect on NA appears to be greater; considering this and the increase in contraction area, it is tempting to speculate on an inhibitory action on the norepinephrine transporter type 1 (NET-1).


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S15. Linking emotional stress to autonomic function
S15.1 The activity of the sympathetic nervous system in conditions characterized by high emotional stress
E. Lambert, T. Dawood, G. Lambert (Human Neurotransmitters Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia)

Psychological factors, including everyday stress, major life events or the presence of depressive illness are now recognized as possible "triggers" for clinical cardiovascular events. Disturbed sympathetic nervous system (SNS) activity could possibly be one underlying mechanism linking psychological stress and cardiovascular disease.

We tested whether subjects with panic disorder and major depressive disorder (MDD) (but free of any cardiovascular conditions) presented with abnormal SNS activity. Microneurographic recordings of multi-unit MSNA in these subjects revealed normal rate of sympathetic nervous activity. However, by applying the technique of single unit MSNA recording (i.e., measuring single vasoconstrictor neuron activity) in subjects with panic disorder and MDD, we noted a disturbed sympathetic firing pattern in that there occurred a higher incidence of multiple firing of vasoconstrictor neurones during a sympathetic burst (increased salvoes). Such an irregular pattern of sympathetic nerve firing may possibly be relevant to the increased cardiac risk as the disturbed pattern of firing was paralleled by an increased release of noradrenaline from the cardiac sympathetic nerves.

We further investigated the link between stress and sympathetic nerve firing pattern in subjects with the metabolic syndrome and hypertension, who commonly, report high level of stress. We examined the SNS activity (multi-unit and single-unit MSNA) in relation to their underlying psychological stress (assessed by the State and Trait Anxiety score) and depression symptoms (assessed by using the Beck Depression Inventory II [BDI]). Multi-unit MSNA was not